The effects of hyperoxia on exercise performance in the cold

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Abstract

This study tested whether hyperoxia improves exercise performance in moderately-cooled individuals, along with the relationship between cold and hyperoxia on cerebral and muscle oxygenation as potential mechanisms for improvement. Twelve healthy trained male cyclists each completed self-paced 15 km time trials (TT) a week apart in three environmental conditions: Neutral (23°C, F_iO_2: 0.21), Cold (0°C, F_iO_2: 0.21), and Cold+Hyper (0°C, F_iO_2: 0.40). Cold conditions were done after participants were passively cooled by 0.5°C rectal temperature. Performance improved with hyperoxia as TT time for Cold+Hyper was faster than Cold, with no difference found compared to Neutral (Neutral: 1479 ± 75s, Cold: 1509 ± 88s, Cold+Hyper: 1482 ± 85s). Cerebral oxygenation in Neutral and Cold+Hyper was higher than Cold throughout the TT, while Cold+Hyper reached similar levels as Neutral by 2.5 km. Improvement in TT time are likely linked to increased O_2 availability allowing for improved aerobic metabolism throughout the body.

Keywords: Pacing, cold stress, hyperoxia, ergogenic aid, voluntary exercise

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List of Abbreviations

ANOVA- Analysis of Variance

BMI- Body Mass Index

CBF- Cerebral Blood Flow

C_aO_2- Arterial Oxygen Content

C_vO_2- Venous Oxygen Content

F_iO_2- Inspiratory Oxygen Fraction

HHb- Deoxygenated Hemoglobin

HR- Heart Rate

MET- Metabolic Equivalent

NIRS- Near Infrared Spectroscopy

O_2- Oxygen

O_2Hb- Oxygenated Hemoglobin

P_aCO_2- Partial Pressure of Carbon Dioxide

P_aO_2- Partial Pressure of Arterial Oxygen

PCU- Partitional Calorimetry Unit

PPO- Peak Power Output

$\dot{Q}$- Maximal Blood Flow

RH- Relative Humidity

RPE- Rating of Perceived Exertion

S_aO_2- Arterial Oxygen Saturation

$Shiv_{peak}$- Peak Shivering

SV- Stroke Volume

TC- Thermal Comfort

ThCOx- Cerebral Oxygen Threshold

tHb- Total Hemoglobin

TOI- Tissue Oxygenation Index

TTE- Time to Exhaustion

TS- Thermal Sensation

TT- Time Trial

T_{re}- Core Temperature

T_{sk}- Skin Temperature

USG- Urine Specific Gravity

$\dot{V}_E$- Ventilation

$\dot{V}O_{2max}$ - Maximal Oxygen Consumption

W- Watts

W_{max}- Maximal Work Rate

Chapter 1: Introduction

Millions of people living in circumpolar regions are regularly exposed to cold environments (0°C to -20°C) throughout much of the year (Reed et al., 2001; Bogoyavlenskiy and Siggner, 2004). Professional and recreational athletes in many sports – such as Nordic skiing and mountaineering – are also exposed to cold environments when training or competing (Oksa et al., 2004). Performing dynamic exercise in these cold environments is physically demanding, with overall performance decreased compared to thermoneutral (~22°C) temperatures (Oksa 2002; Oksa et al., 2004; Nimmo, 2004; Castellani et al., 2006; Racinais and Oksa, 2010). Aside from athletics, individuals can encounter environmentally stressful situations during occupational activities (Oksa et al., 1997) including resource industries (e.g., fishing, petroleum extraction) and all-season occupations such as construction and in the military (Cheung et al., 2015). Likewise, cold stress can occur in indoor settings, such as manufacturing and food processing. Depending on occupations, like in the mining industry, construction work, agriculture, forestry or seafaring, cold exposure may be considerable, lasting for several hours at a time (Cheung et al., 2015), while military personnel may also frequently be faced with harsh environmental conditions during training or deployment in the field.

A combination of low temperatures, passive exposure, or inadequate clothing can result in cooling of the tissues. This leads to decrements in dynamic exercise performance which have been attributed to impaired mechanisms such as cardiorespiratory hemodynamics, oxygenation to the brain and muscle tissues, and mechanical/metabolic inefficiency of the muscle (Bergh and Ekblom, 1979; Blomstrand and Essen-Gustavsson,

1987; Nimmo 2004; Oksa, 2002; Oksa et al., 2004; Quirion et al., 1989). Primarily, peripheral vasoconstriction shifts blood from the peripheries to the core in order to maintain core temperature and keep vital organs safe. As a result, both cerebral and muscle blood flow have been shown to decrease in the cold (Minett et al., 2014 Rowell, 1974 & 2011; Pendergast 1988; Johnson et al., 2014). Reductions in blood flow to cerebral and muscle tissue will likely decrease the amount of O_2 delivered to these tissues unless oxygen (O_2) extraction increases in compensation (Blomstrand and Essen-Gustavsson, 1987; Pendergast 1988; Quirion et al. 1989). Ultimately, reduced blood flow and O_2 delivery to the active tissue has downstream effects during exercise leading to increased rates of fatigue, thus increasing physical strain compared to a thermoneutral environment leading to decreased physical performance, occupational inefficiency, and increased risk of accidents (Oksa et al., 1997; Oksa et al., 1998).

Hyperoxia has been used extensively in thermoneutral environments to improve dynamic exercise performance (Knight et al., 1993; Linossier et al., 2000; Eves et al., 2002a,b; Amann et al., 2006; Tucker et al., 2007) through increasing arterial oxygen content (C_aO_2) within the body (Ekblom et al., 1975; Knight et al., 1993; Nielsen et al., 1998; Nielsen et al., 1999; Richardson et al., 1999; Dempsey and Wagner 1999). Increasing C_aO_2 allows for greater O_2 availability in the body, which is the primary proposed mechanism for exercise improvement with hyperoxia (Welch, 1981). Greater O_2 availability could potentially lead to improvements in cardiorespiratory hemodynamics, oxygenation to the brain and muscle tissues, and mechanical/metabolic efficiency of the muscle. For example, hyperoxia has been shown to reverse cerebral deoxygenation that normally occurs during intense exercise in normoxia (Nielsen et al.,

1999; Nybo and Rasmussen, 2007). This is of importance as cerebral hypoxemia has been shown to play a role in altering central motor drive (Tucker et al. 2007). Whether hyperoxia has a similar response in the muscle is unclear as Nielsen et al. (1999) and Oussaidene et al. (2013) showed no change in the level of oxygenation in the muscle with hyperoxia (F_iO_2: 0.30) despite an increase in C_aO_2 of 4-7%. The rate of accumulation of fatigue related metabolites has also been shown to decrease with hyperoxia (Hogan et al. 1999).

Ultimately hyperoxia increases the O_2 content within the body, allowing for greater O_2 availability which seems to improve aerobic efficiency, resulting in improved exercise performance. Thus, it is possible that hyperoxia could counteract the systemic vasoconstriction and impaired tissue oxygenation observed with mild body cooling. Therefore, the purpose of this study was to determine if hyperoxia could improve dynamic exercise performance in mildly-cooled individuals, and investigate potential mechanisms for improvement, specifically cerebral and muscle oxygenation. It was hypothesized that: 1) 15 km cycling time trial (TT) performance in Neutral would be quicker than with moderate (-0.5°C) cooling during exercise in both Cold and Cold+Hyper trials, with Cold+Hyper improved compared to Cold. 2) Cold+Hyper and Neutral would have enhanced cerebral oxygenation compared to Cold, while muscle oxygenation would be maintained across conditions.

Chapter 2: Literature Review

What defines cold depends on the perspective from which it is examined. From a physiological point of view, cold could be an environmental temperature activating heat-conserving mechanisms such as cutaneous vasoconstriction or increased metabolic heat production (shivering) (Johnson et al., 2014) to maintain a core temperature of ~37°C. However, from a behavioral perspective cold could be any ambient temperature below 20°C where unsafe occupational behavior starts to increase (Ramsey et al. 1983). Regular temperatures winter athletes can face range between 10°C to -20°C (Sandsund et al. 2012) while ambient winter temperatures for occupations such as mining or military field training can range between 0°C to -29°C (Rintamäki et al., 2004). Indoor cold working environments are especially prevalent in the food industry as well, from the actual processing of meats and seafood occurring in ambient temperatures of 0°C to 10°C and in cold storage at temperatures below -20°C (Mäkinen and Hassi, 2009). In these field and occupational settings core temperature has been found to be as low as 36.3°C (Rintamäki et al., 2004; Oliveira et al., 2014). Despite these regularly occurring temperatures, athletes or occupational workers in these environments must take into consideration the consequences of cold on performance.

2.1 Cold and Exercise Performance

Dynamic exercise performance is decreased when exposed to cold environments either acutely or chronically (Patton and Vogel, 1984; Quirion et al., 1989; Oksa and Rintamäki 1995; Oksa 2002; Oksa et al., 2004; Nimmo 2004; Oksa et al., 2010; Sandsund et al., 2012; Wiggen et al., 2013) more so than isometric exercise (Oksa, 2002; Oksa et al., 2010). Dynamic performances, measured by time to exhaustion (TTE) tests,

have shown decrements in performance up to 38% across varying ambient temperatures (Patton and Vogel, 1984; Oksa et al. 2004; Sandsund et al, 2012; Wiggen et al., 2013). Decrements were shown in TTE at 75-80% $\dot{V}O_{2max}$ following 18-24 h exposure at 20°C and -20°C, with a reduction of 38% in TTE (66.9 ± 13.6 min vs. 111.9 ± 22.8 min) occurring at -20°C compared to 20°C (Patton and Vogel 1984). Sandsund et al. (2012) tested TTE across 6 ambient temperatures (-14°C, -9°C, -4°C, 1°C, 10°C, 20°C), finding significant decrements in -9°C and -14°C, thus expanding on the range of temperatures from Patton and Vogel's (1984) work in which performance is decreased. Table 1 summarizes performance decrements in the cold.

With acute exposure, there seems to be an "inverted U" relationship between ambient temperatures and performance. Galloway and Maughan (1997) compared exercise at 70% $\dot{V}O_{2max}$ in ambient temperatures of 4°C, 11°C, 21°C, and 31°C during a cycling TTE exercise. TTE was greatest (93.5 min) at 11°C and shortest (51.6 min) at 31°C with no significant differences between 4°C (81.4 min) and 21°C (81.2 min). Sandsund et al. (2012) found TTE running performance to improve the most at 1°C and -4°C compared to ambient temperatures between 20°C and -14°C. Therefore, it seems that ambient temperatures ranging from 11°C to -4°C improve and/or maintain various dynamic exercise performance when exposure before exercise is minimal while ambient temperatures below -4°C decreases performance.

Table 1 Overview of Exercise Performance in Cold. Maximal oxygen uptake ($\dot{V}O_{2max}$); Time to exhaustion (TTE).

Reference	Environmental Condition	Exercise Test	Exercise Duration	Outcome
Oksa et al., 2004	20, 0, -10, -20°C	Treadmill $\dot{V}O_{2max}$	~20 minutes	↑ $\dot{V}O_{2max}$
Crowley et al., 1991	Water immersion 11.5°C	Wingate	30 seconds	↑ Power Output
Bergh and Ekblom, 1979a	Water Immersion	Cycling Sprints	<30seconds	↑ Power output
Bergh and Ekblom, 1979b	Water Immersion 13-15°C	Cycling TTE	~5min	↑ TTE
Blomstrand et al., 1984	Water Immersion 10-12°C	Cycling TTE	~2min	↑ TTE
Patton and Vogel, 1984	20C and -20°C	Cycling TTE	>60mins	↑ TTE
Blomstrand and Essen-Gustavsson, 1987	Water Immersion 10-12°C	Cycling TTE	<2 mins	↑ TTE
Quirion et al., 1989	20, 10, 1, -4, -9, -14°C	Cycling TTE	-	↑ TTE ↑ $\dot{V}O_{2max}$ ↑ Power Output
Sandsund et al., 2012	20, 10, 1, -4, -9, -14°C	Running TTE	-	↑ TTE
Wiggen et al., 2013	6 and -14°C	Running TTE	~5min	↑ $\dot{V}O_{2max}$ ↑Power Output

Exercise duration will likely be a factor in determining the performance decrement seen across experiments as well. These decrements are likely due to several functional properties of the muscle that are affected by cooling (Faulkner et al., 1990), which are discussed later in this review. Exercises that are very fast, intense, and rely on the elastic properties of the muscles are more susceptible to cooling than exercises of longer duration (Oksa et al., 1997). Table 2 summarizes performance decrements in relation to exercise duration.

Table 2 Duration of Exercise and Performance Decrements.

Duration of Exercise	Reduction in Performance (%)
1-2s	6-31
3-15s	6-20
16-60s	11-22
1-15min	13-18
Hours	10-28
Days	4-10

Reference used: Oksa et al. (1997)

2.1.2 Mechanisms

Fatigue is a multi-modal phenomenon (Enoka, 1992), thus this section will focus on three specific mechanisms affected in the cold: changes in hemodynamics, cerebral/muscle oxygenation, and functional properties of the muscle. Exposure to the cold causes peripheral vasoconstriction, which reduces cerebral and muscle blood flow. Reduced blood flow decreases O_2 delivery to cerebral and muscle tissue therefore causing decrements in performance by contributing to fatigue. Functional properties of the muscle are also affected by the actual cooling of the muscle further contributing to mechanical

inefficiency, increased rates of fatigue, and decreased performance. Figure 1 shows the inter-relationships between these mechanisms during exercise in the cold.

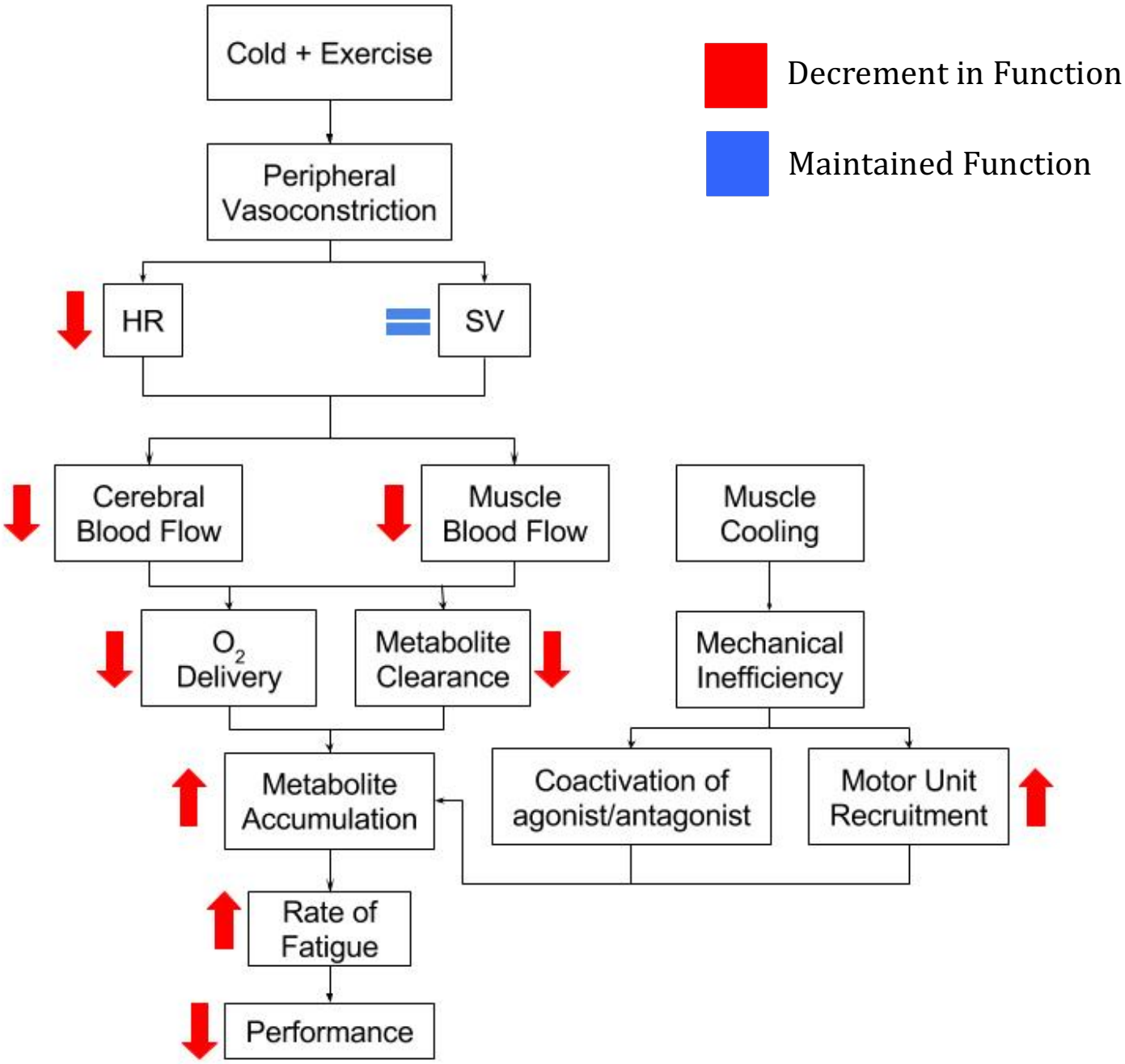


Figure 1 Interrelationship Between Mechanisms During Exercise in the Cold. Heart rate (HR); Oxygen (O_2); Stroke volume (SV).

2.1.3 Cardiovascular/Hemodynamics

The primary cardiovascular response seen in the cold is decreased heart rate (Berg and Ekblom, 1979; Berg and Ekblom, 1987; Shiojiri et al. 1997; Nimmo 2004; Oksa et al., 2004; Sandsund et al., 2012; Kean et al., 2015). The primary contributing factor is peripheral vasoconstriction causing a shift in blood to the core rather than the peripheries in order to maintain a core temperature ~37°C (Beelan and Sargeant, 1991; Flore et al. 1992; Nimmo 2004; Kean et al., 2015). The redistribution of blood increases central venous pressure, total peripheral resistance, and diastolic blood pressure causing a baroreceptor-mediated stimulus that results in a reflex slowing of heart rate (Beelan and Sargeant, 1991; Shiojiri et al., 1997; Nimmo 2004). These changes will cause an overall decrease in blood flow, which can have additional implications on exercise performance such as decreased O_2 delivery.

2.1.4 Muscle and Cerebral Blood Flow/Oxygenation

Reductions in blood flow to skeletal muscle and cerebral tissue can decrease the amount of O_2 delivered to these tissues (Blomstrand and Essen-Gustavsson, 1987; Pendergast 1988; Quirion et al. 1989) unless O_2 extraction increases in compensation. Limb blood flow has been shown to decrease compared to thermoneutral environments as a result of cold induced vasoconstriction, and can approach 0 with a core temperature of 36°C (Rowell, 1974 & 2011; Pendergast 1988; Ishii et al., 1992; Shiojiri et al., 1997; Ihsan et al., 2013; Johnson et al., 2014) while cerebral blood flow has been shown to decrease with cold water immersion (Minett et al., 2014). Ambient temperatures of 10°C have been shown to reduce cerebral oxygenation by 9% during passive exposure in a supine position (Kean et al., 2015) while Minett et al. (2014) also showed that cerebral

oxygenation is reduced with acute cold water immersion. This becomes problematic when you consider the Fick principle:

$$\dot{V}O_2 = \dot{Q} \times (C_aO_2 - C_vO_2)$$

where $\dot{Q}$ is blood flow and $C_aO_2 - C_vO_2$ is the difference in arterial and venous O_2 concentration.

Since peripheral vasoconstriction reduces blood flow in the cold, it is likely that there is an O_2 supply limitation from blood flow occurring, which could explain reduced exercise performances.

While cold environments decrease blood flow and oxygenation to the muscle and cerebral tissue, a temperature-related shift in the O_2-dissociation curve can also reduce O_2 extraction at the tissues (Bergh and Ekblom, 1979b; Beelen and Sargeant, 1991; Shiojiri et al., 1997). When blood temperature decreases below 37°C, less O_2 is delivered to skeletal and cerebral tissues due to a change in hemoglobin's molecular structure, which binds to O_2 molecules more tightly thus shifting the curve up and to the left (Bergh and Ekblom, 1979). Therefore, the slower arterial mixed venous O_2 difference response to exercise in the cold probably reflects poor O_2 extraction in muscle as well as reduced muscle blood flow caused by cold-induced vasoconstriction (Shiojiri et al., 1997). However, Bergh and Ekblom (1979a) stated that a temperature difference of 3-4°C is not likely to change diffusion to an extent that would induce any measurable effect on $\dot{V}O_2$ during maximal exercise. They argue that other peripheral factors, like local blood flow and enzyme activities, are a more likely cause of reduced performance at lower body temperatures.

Reductions in blood flow and O_2 delivery to muscle and cerebral tissues will decrease the effectiveness of oxidative pathways, thus relying on anaerobic contributions (Bergh and Ekblom, 1987; Weller et al. 1997). Oxidative reactions providing energy for exercise in cold muscles may also be slowed by the temperature-dependent reduction of enzyme activity and/or by poor O_2 supply due to a reduced diffusion rate, compensated for by the anaerobic process (Shiojiri et al., 1997). Increased anaerobic metabolism consequently increases lactate levels in the blood and muscle, therefore increasing the rate of fatigue and decreasing the ability to sustain exercise performance.

2.1.5 Mechanical and Metabolic Inefficiency

Cooling of the muscles will affect their functional properties, thus contributing to mechanical inefficiency and reducing performance. Dynamic muscular performance can be broken down into several components: endurance, force, power, velocity, and coordination, which are all negatively affected by cooling (Oksa and Rintamäki, 1995; Oksa, 2002; Racinais and Oksa, 2010; Oksa et al., 2010). Dynamic muscular performance has been shown to have a dose-response relationship with a drop between 2-10% per °C decrease in muscle temperature (Berg and Ekblom, 1979; Sargeant 1987; Oksa and Rintamäki, 1995; Oksa, 2002; Oksa et al., 2010). Significantly higher decreases of 17% per °C have been shown in drop jump exercise, indicating that tasks that require fast muscular movements are most susceptible to cooling (Oksa et al., 1997).

These performance components are likely affected due to several mechanical, neural, and biochemical functional properties (Crowley et al., 1991; Oksa and Rintamäki, 1995; Oksa et al., 1997; Oksa, 2002). Several studies have shown that when muscle temperature decreases, co-activation of the agonist and antagonist muscles occur (Bawa

et al., 1987; Oksa et al., 2002). Similarly, Oksa et al. (1995, 1997) have reported that, during the concentric phase of contraction in a drop-jump, the activity of the agonist muscle decreases and at the same time the activity of the antagonist muscle increases. The authors term this the "braking effect" which leads to a decreased muscular performance. This co-activation not only causes the muscles to fight against each other, but also causes an increase in muscle recruitment all of which contribute to muscular inefficiency and increased O_2 consumption (Bawa et al., 1987; Oksa, 2002; Oksa et al., 2004).

Neural mechanisms that are affected by cooling are slower muscle and nerve conduction velocities as well as altered motor unit recruitment (Denys, 1991; Oksa et al., 2010). Slowing of nerve conduction velocity with cooling will induce slower and weaker muscle contractions. However, the reduced nerve conduction velocity in cold environments may result in an increased temporal summation, leading to an increase in motor unit recruitment in order to maintain the same amount of work (Racinais and Oksa, 2010) as shown by increases in EMG activity (Oksa et al., 2002).

Several biochemical properties affected by cooling are: slowing down of chemical reactions in the muscle, delays in the cross-bridge cycle, and decreased acto-myosin sensitivity to calcium (Ishi et al. 1992; Shiojiri et al., 1997; Racinais and Oksa, 2010). Decrements in any of these functional properties ultimately will enhance accumulation of lactate within the muscle (Blomstrand and Essen-Gustavsson, 1987; Beelen and Sargeant, 1991; Shiojiri et al., 1997; Wiggen et al., 2013) therefore contributing to increased rates of fatigue and decreased performance.

2.2 Hyperoxia and Exercise Performance

Dynamic exercise performance is improved with the use of hyperoxia as shown by improved maximal aerobic capacity (Ekblom et al., 1975; Welch 1981; Knight et al., 1993; Nielsen et al., 1998), along with performance through both TTE (Linossier et al., 2000; Amann et al., 2006; Tucker et al., 2007) and time trial (TT) tests (Knight et al., 1993; Peltonen et al., 1995; Richardson et al., 1999; Linossier et al., 2000; Petersen et al., 2000; Eves et al., 2002a; Eves et al., 2002b; Amann et al., 2006; Tucker et al., 2007; Oussaidene et al., 2013). Amann et al. (2006) found that 5 km TT performances improved by 3-6% with an inspiratory O_2 fraction (F_iO_2) of 1.0 compared to FiO_2 values of 0.15, 0.21 and 0.24, while also showing an increase in average power output for the duration of the TT's as characterized by a higher plateau, and greater rise in power output compared to the other F_iO_2 conditions. Similarly, Tucker et al. (2007) showed that 20 km TT performance improved by 5% with a F_iO_2 of 0.40, indicating that 100% O_2 is not necessary to see improvements in performance. Exercise improvements with hyperoxia have been observed across $F_iO_2$values between 0.26 and 1.0, while a F_iO_2 of 0.30 has shown to attenuate the decrements seen in arterial O_2 saturation during intense exercise and increase maximal oxygen consumption ($\dot{V}O_{2max}$) (Nielsen et al., 1998). Arterial O_2 content (C_aO_2) is a product of the amount of O_2 bound to hemoglobin and the amount of O_2 dissolved in the blood, thus one can think of C_aO_2 as a representation of the amount of O_2 available for delivery to working tissues. Overall hyperoxia seems to improve performance by increasing C_aO_2 therefore increasing O_2 supply in the body.

Table 3 Overview of Hyperoxia and Exercise Performance. Inspiratory oxygen fraction (F_iO_2); Maximal oxygen consumption ($\dot{V}O_{2max}$); Time to exhaustion (TTE); Time trial (TT); Maximal work rate (W_{max}).

Reference	F_iO_2	Exercise Test	Exercise Duration	Outcome
Petersen et al., 2000	0.4	Firefighting circuit	~5min	↑ Time to completion
Eves et al., 2002a	0.4	Treadmill graded exercise test	-	↑ $\dot{V}O_{2max}$ ↑ Power Output
Peltonen et al. 2001	0.32	Maximal Cycling test	7 minutes	↑ $\dot{V}O_{2max}$
Knight et al., 1993	1.0	Incremental Cycle exercise	~4 min	↑ Leg $\dot{V}O_{2max}$ ↑ Max Work Rate
Nielsen et al., 1998	0.3	Rowing Ergometer	6 minutes	↑ $\dot{V}O_{2max}$
Peltonen et al., 1995	0.62	Rowing: TT	2500m	↑ $\dot{V}O_{2max}$ Improved TT
Nielsen et al., 1999	0.3	Rowing: TT	6 minutes	↑ $\dot{V}O_{2max}$ ↑Work
Ekblom et al., 1975	0.5	Treadmill: TTE	6 minutes	↑ $\dot{V}O_{2max}$
Richardson et al., 1999	1.0	Cycling: TTE	~6minutes	↑ TTE ↑ W_{max}
Amann et al., 2006b	1.0	Cycling: 5km TT	~8 minutes	Improved TT ↑ Power Output
		Cycling: TTE	-	↑ TTE
Tucker et al., 2007	0.4	Cycling: 20km TT	~30 minutes	Improved TT ↑ Power Output
		Cycling: TTE	~30 minutes	↑ Power output ↑ W_{max}

2.2.1 Mechanisms

Hyperoxia increases C_aO_2 in the body (Ekblom et al., 1975; Knight et al., 1993; Nielsen et al., 1998; Nielsen et al., 1999; Richardson et al., 1999; Dempsey and Wagner 1999; Linossier et al. 2000; Eves et al., 2002a; Amann et al. 2006), however, the mechanisms behind how C_aO_2 improve exercise performance remain unclear (Linossier et al., 2000; Petersen et al., 2000; Amann et al., 2006). This section will focus on three specific mechanisms that increased C_aO_2 is thought to affect: changes in cerebral blood flow/oxygenation, muscle blood flow/oxygenation, and aerobic metabolism. Increases in blood flow or oxygenation in either the brain or muscle can help improve O_2 delivery allowing performance to improve, while greater reliance on aerobic metabolism results in mechanical efficiency thus decreasing rate of fatigue development. Figure 2 shows the inter-relationships of these mechanisms during hyperoxia and exercise.

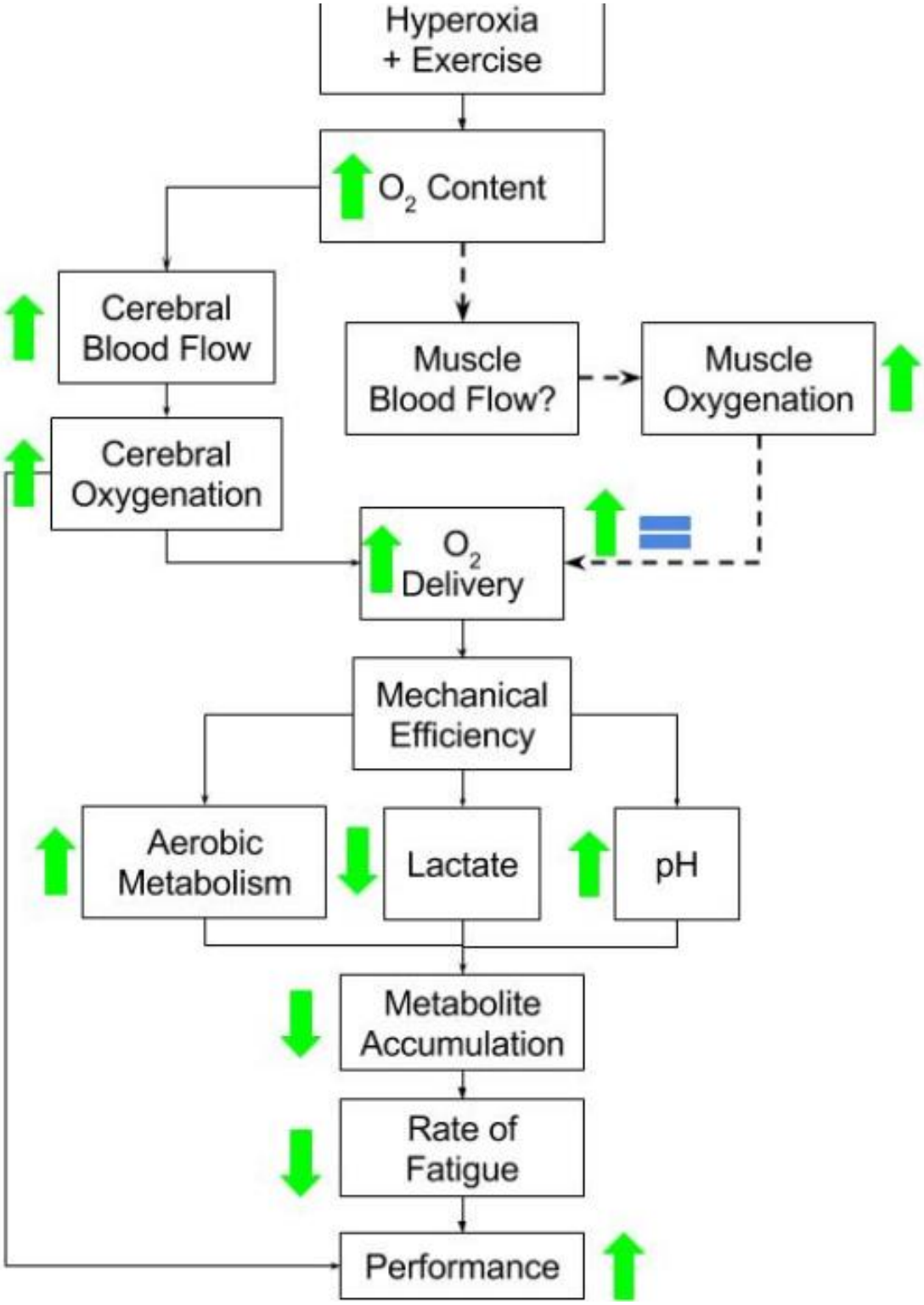


Figure 2 Mechanisms Affected During Hyperoxia and Exercise. Oxygen (O_2); The green arrows indicate where hyperoxia improves mechanisms. The dashed line represents an unknown effect in terms of improvement or decrement in oxygen delivery.

2.2.2 Cerebral Blood Flow/Oxygenation

Brain perfusion is highly sensitive to changes in the partial pressures of carbon dioxide in arterial blood (P_aCO_2) and, to a lesser degree, the partial pressure of O_2 in arterial blood (P_aO_2) (Ainslie and Duffin, 2009; Smith et al., 2012; Willie et al. 2012). Elevations in P_aCO_2 (hypercapnia) lead to vasodilation of cerebral arterioles causing a subsequent increase in cerebral blood flow (CBF), while a reduction in P_aCO_2 (hypocapnia) leads to vasoconstriction and a subsequent decrease in CBF (Ainslie and Duffin, 2009). This change in CBF in response to changes in P_aCO_2 is termed CO_2 reactivity, which is a homeostatic function that is important to regulate and maintain central pH by removing waste products from the cerebral metabolism (Nybo and Rasmussen, 2007; Ainslie and Duffin, 2009) as well as providing O_2 to the tissue (Nybo and Rasmussen, 2007). CBF is decreased with the administration of hyperoxia during rest (Watson et al., 2000; Willie et al., 2012), as a result of changes in P_aCO_2. Since hyperoxia increases C_aO_2 there is a decreased P_aCO_2 indicating that the brain is hypocapnic resulting in cerebral vasoconstriction, which is thought to occur due to the pial arteries (Watson et al., 2000) further supporting the idea that P_aCO_2 is the primary force driving changes in CBF.

However, Willie et al. (2012) showed regional differences in the cerebrovascular response to changes in arterial blood gases, which were shown by differences between the neck (vertebral and internal carotid) and cerebral (middle cerebral and posterior cerebral) arteries indicating that with severe changes in arterial blood gases the large vessels of the neck are not mere conduit vessels, and rather act as active resistance vessels. These differences in flow to the brainstem and cortex that were found challenge

the long-held paradigm that cerebrovascular resistance is solely modulated at the level of the arteriolar pial vessels (Willie et al., 2012). Regional changes should also be considered for future studies examining cerebrovascular function since these differences may cause under or overestimations of cerebrovascular variables (Willie et al., 2012). Regional differences were further supported by the findings of Smith et al. (2012) who showed that middle cerebral artery velocity was maintained while posterior cerebral artery velocity was significantly elevated with hyperoxia during mild intensity exercise. This is a significant finding since this is the first time that hyperoxia has been shown to have a regional difference on CBF (Smith et al., 2012). The latest work by Smith et al. (2016) shows that global CBF increases with hyperoxia, indicating that CBF regulation at least during submaximal exercise is not solely dependent on P_aCO_2, and could rely more on cardiac output, cerebral metabolism, or cerebral perfusion pressure. Thus, is seems that while there are regional differences in CBF, hyperoxia appears to enhance global CBF, allowing for greater removal of metabolites and maintenance of cerebral oxygenation.

Maintenance of cerebral oxygenation with hyperoxia is important since cerebral hypoxemia has shown to decrease exercise performance (Nybo and Rasmussen, 2007; Amann and Calbet 2008; Subudhi et al., 2008; Ainslie and Duffin, 2009; Miyazawa et al., 2013; Oussaidene et al., 2013). Increasing C_aO_2 with hyperoxia has been shown to reverse cerebral deoxygenation that normally occurs during intense exercise in normoxia, which is shown by a maintained cerebral oxygenation level to that of resting values during exercise (Nielsen et al., 1999; Nybo and Rasmussen, 2007). Amann et al. (2007) showed that physical fatigue during acute exposure to severe hypoxia (F_iO_2: 0.10) was

largely influenced by central factors, since a rapid switch to hyperoxia (F_iO_2: 0.60) improved cerebral oxygenation and prolonged TTE, in the absence of a critical level of peripheral muscle fatigue. Similarly, Subudhi et al. (2008) showed that hyperoxic gas (F_iO_2: 0.60) administered at maximal exertion during acute hypoxia increased cerebral oxygenation above resting values and increased pedal cadence, allowing a similar maximal work rate to be achieved compared to normoxia. In other words, the administration of hyperoxia alleviated cerebral hypoxemia allowing subjects to continue the graded exercise test and reach similar maximal work rates as achieved in normoxia. This immediate improvement in performance is likely due directly to reversal of cerebral deoxygenation rather than changes in muscle oxygenation, since the effect on performance were too quick to have been mediated by a reduction in peripheral muscle fatigue (Subudhi et al., 2008). Oussaidene et al. (2013) showed cerebral oxygenation decreased in both normoxia and hyperoxia during TTE, however hyperoxia delayed the O_2 supply and demand imbalance as shown by the higher maximal work rate achieved in hyperoxia compared to normoxia before exercise was terminated. Oussaidene et al. (2013) attributes this to the increased O_2 supply from hyperoxia, which delayed the time until the cerebral oxygenation threshold (ThCOx) (imbalance between O_2 supply and O_2 demand of the brain) was reached. Together, these studies not only show that hyperoxia increases cerebral oxygenation, but also suggest that there may be a hypoxemia-sensitive up or down regulation of central motor output outside of any peripheral muscle fatigue and its associated afferent feedback, which could explain the improved cycling cadence or increased power output with hyperoxia.

2.2.3 Muscle Blood Flow/Oxygenation

Hyperoxia can decrease muscle blood flow during rest and exercise (Welch et al., 1977; Stellingwerff et al., 2006; Casey et al., 2011; Casey and Joyner, 2011), however opposing results have shown leg blood flow to be maintained (Knight et al., 1993; Richardson et al., 1999). Casey et al. (2011) mentions that the mechanisms behind reduced limb blood flow are still unclear, but may be due to the direct vasoconstrictor action of O_2 on the arterial and arteriolar wall. Accumulating evidence suggests that erythrocytes have the ability to sense changes in O_2 as well as modulate vascular tone via the release of ATP or nitric oxide, thus leading to appropriate changes in blood flow and matching O_2 delivery with metabolic need (Casey et al., 2011). Therefore, decreasing the release of ATP/nitric oxide in response to increased C_aO_2 could potentially be the reason for reduced limb blood flow during exercise (Casey et al., 2011; Casey and Joyner, 2011).

While reduced limb blood flow would reduce O_2 delivery to the muscle in normoxia one must first consider the Fick principle again. Therefore, with hyperoxia maximal O_2 extraction in the muscle would decrease due to reductions in limb blood flow according to this principle, however increased C_aO_2 within the body would compensate for these reductions, thus allowing for maintained O_2 extraction and meeting O_2 demands. Stellingwerff et al. (2006) also proposed that a reduced leg blood flow during hyperoxia would result in a longer red cell transit time, thus reducing O_2 diffusion limitations and allowing for maintained O_2 extraction to occur anyways. However, if muscle blood flow is not reduced we can look at the Fick principle and see that maintenance in blood flow

and improvement in oxygenation would increase maximal O_2 uptake, which could explain improved performances.

Whether or not hyperoxia improves O_2 delivery to the muscle is unclear. Nielsen et al. (1999) and Oussaidene et al. (2013) showed no change in the level of oxygenation in the muscle with hyperoxia (F_iO_2: 0.30) as shown by near infrared spectroscopy (NIRS) despite an increase in C_aO_2 of 4-7%. However, Oussaidene et al. (2013) suggests if one considers that deoxygenated hemoglobin (HHb) represents the O_2 utilization/O_2 delivery ratio in the active muscle and C_aO_2 increased 4-7% with hyperoxia, we may conclude that the unchanged muscle HHb at maximal exercise could mean that the muscle utilized more O_2 under hyperoxia. Knight et al. (1999) showed that O_2 supply is a limiting factor of maximal exercise performance. Compared to normoxia, hyperoxia (F_iO_2: 1.0) caused an 8.1% increase in leg VO_2max and increased maximal O_2 delivery by 10.9% during cycling exercise. The authors noted that if blood flow increased as well as C_aO_2 and there was not an increase in leg $\dot{V}O_{2max}$there would be indications that exercise is limited by O_2 demand. However, since leg $\dot{V}O_{2max}$ increased from the changes in C_aO_2 the authors concluded that leg $\dot{V}O_{2max}$ is limited by O_2 supply and showing the importance of maintaining oxygenation during exercise. Similarly, Richardson et al. (1998) showed increased O_2 delivery to the leg muscle during knee extension exercise when using a F_iO_2 of 1.0. Results showed increases in leg $\dot{V}O_{2max}$ compared to normoxia, again showing that an O_2 supply limitation seems to be causing decreases in performance. This was further displayed by the author's results showing that the diffusion capacity in the leg across F_iO_2 conditions was maintained indicating that mitochondrial metabolism of the muscle wasn't limiting exercise performance. Overall the literature seems to indicate that

in normoxia there is a clear O_2 supply limitation that leads to performance decrements (Basset and Howley, 2000) and the use of hyperoxia can improve and/or maintain O_2 delivery to the muscle therefore possibly explaining the improvements in exercise performance.

2.2.4 Mechanical and Metabolic Efficiency

Increased blood flow/oxygenation of the cerebral tissue and increased/maintained oxygenation to muscle tissues with hyperoxia has been shown to affect some biochemical properties within the body, specifically decreased pyruvate accumulation (Stellingwerff et al., 2006), decreased blood/muscle lactate levels (Peltonen et al., 1995; Linossier et al., 2000; Eves et al., 2002b; Amann et al., 2006; Romer et al., 2006; Stellingwerff et al., 2006), decreased P_i accumulation (Amann et al., 2006b), and increased pH (Peltonen et al., 1995; Linossier et al., 2000; Stellingwerff et al., 2006). All together these changes decrease the biochemical and physiological disturbances to homeostasis by allowing greater reliance on aerobic metabolism, thus allowing for mechanical efficiency by reducing fatigue development. Amann et al. (2006) found peripheral fatigue measurements across F_iO_2 conditions of 0.15, 0.21, 0.24, and 1.0 to be identical at the end of the 5km TT and TTE tests, however both tests significantly improved with an F_iO_2 of 1.0 indicating that hyperoxia had slowed the rate of peripheral fatigue. Reduced rates of various metabolites are of great importance since group III and IV muscle afferents innervate free nerve endings distributed widely throughout the muscle. Metabolic byproducts of muscular contractions such as H^+ and P_i have been shown to increase the spontaneous discharge of both group III and IV afferents, therefore sending inhibitory feedback to the central nervous system and reducing central motor drive (Amann et al.,

2006; Nybo and Rasmussen, 2007; Amann and Calbet, 2008). In conclusion, hyperoxia may improve biochemical processes by relying on aerobic metabolism rather than anaerobic metabolism, causing reduced metabolite accumulation along with reducing rates of peripheral fatigue.

2.3 Cold + Hyperoxia

The effect of hyperoxia on exercise in the cold has not previously been studied to the author's knowledge. The novelty of this study lies in the use of hyperoxia as an ergogenic aid in the cold, thus acting as a means to improve physical performance, occupational efficiency, and decrease the risk of accidents for numerous individuals exposed to cold climates such as those in artic and circumpolar regions.

Since there is no body of literature combining cold and hyperoxia together, the outcomes of the experiment can only be speculated from the previous findings of cold and hyperoxic environments on exercise performance separately. In the cold it is well known that decrements in dynamic exercise performance have been attributed to several mechanisms such as cardiorespiratory hemodynamics, oxygenation to brain and muscle tissues, and mechanical/metabolic inefficiency. Thus, hyperoxia is likely to work by increasing C_aO_2 in the body during exercise in the cold, targeting the oxygenation aspect of the decrements seen in the cold. Increasing C_aO_2 will increase cerebral and either improve or maintain muscle oxygenation during exercise, hopefully having a downstream effect on performance by enhancing aerobic metabolism and decreasing metabolite accumulation resulting in reduced rates of fatigue and increased performance. In conclusion, we suspect hyperoxia to counteract the decrements in performance that occur

in the cold. Figure 3 shows the potential interaction that hyperoxia will have on exercise in the cold.

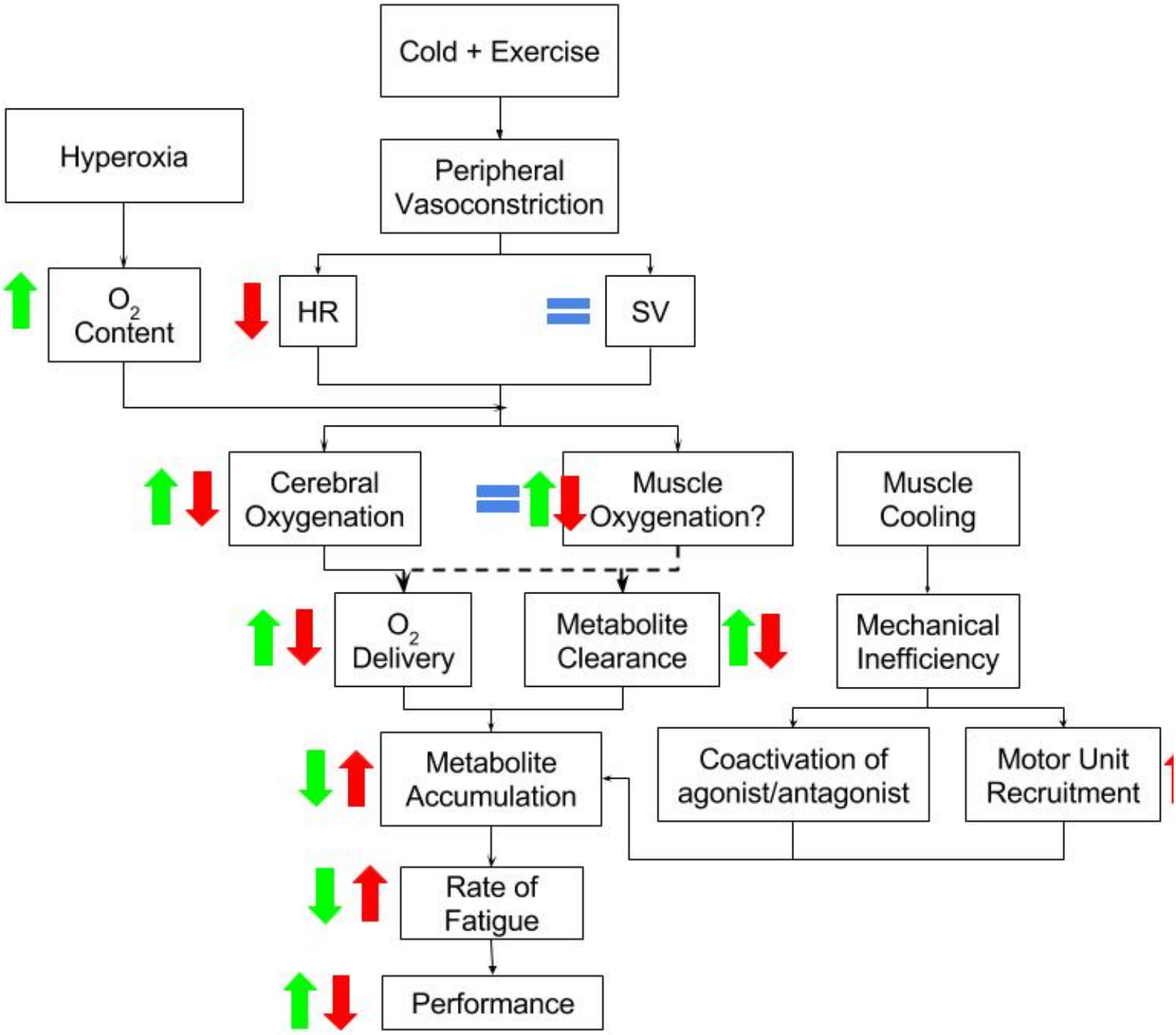


Figure 3 Potential Interactions. The red arrows represent decrements in mechanisms from the cold while the green arrows represent improvements in mechanisms with hyperoxia. Oxygen (O_2); Heart rate (HR); Stroke volume (SV).

Chapter 3: Objectives and Hypotheses

3.1 Objectives

The objectives of this study were to:

1. Determine if hyperoxia can improve exercise performance in mildly-cooled individuals.

2. Investigate potential mechanisms for improvement, specifically cerebral and muscle oxygenation.

3.2 Hypotheses

The hypotheses of this study were:

1. 15 km cycling time trial (TT) performance in Neutral would be quicker than with moderate (-0.5°C) cooling during exercise in both Cold and Cold+Hyper trials, with Cold+Hyper improved compared to Cold.

2. Cold+Hyper and Neutral would have enhanced cerebral oxygenation compared to Cold, while muscle oxygenation would be maintained across conditions.

Chapter 4: Methods

4.1 Participants

The study was approved by Brock University's Bioscience Research Ethics Board (BREB #16-017) and conformed to the standards set by the Declaration of Helsinki. All participants were screened using a modified Physical Activity Readiness Questionnaire (PAR-Q) and were informed of the experimental protocol and associated risks prior to participating in the experiment. Verbal and written consent was obtained from each participant.

Twelve healthy, trained male cyclists were recruited from the university and general public for this experiment. Participants were non-smokers, and were free from cardiovascular, neurological, and skeletal disorders based on the PAR-Q form. The mean (± SD) for age, height, mass, body fat percentage, peak oxygen consumption, and peak power output was 29.8 ± 8.1 years, 177.6 ± 6.0 cm, 73.5 ± 7.3 kg, 11.2 ± 4.1 %, 74.9 ± 8.0 ml/(kg·min), and 395.5 ± 42.2 watts, respectively. Based on DePauw et al. (2013), participants were classified as performance level 3-4 (scale of 1-5).

4.2 Experimental Design

Participants underwent one familiarization and three experimental sessions for a total of four lab sessions. The familiarization session consisted of collecting anthropometric data, completing a $\dot{V}O_{2peak}$ test on a cycle ergometer, and a 15 km cycling TT. Sessions 2-4 are the experimental sessions, which were done in a randomized order for each participant. The experimental sessions consisted of a 15 km TT in different environmental conditions: 2) Neutral (23°C, F_iO_2: 0.21), 3) Cold (0°C, F_iO_2: 0.21), and 4)

Cold+Hyper (0°C, F_iO_2: 0.40). Sessions 2-4 were separated by a minimum of 1 week to ensure proper recovery time and reduce the potential effects of cold acclimation.

4.2.1 Familiarization

Participant's age (years) as well as their height (cm) and mass (kg) were measured upon arrival. To determine body density, skin fold thickness was measured at seven sites (triceps, sub-scapula, abdomen, supra-iliac crest, mid-axilla, thigh, and pectoralis major) (Jackson & Pollack, 1978) with manual calipers (Harpenden, Bay International, West Sussex, UK). Percent body fat was calculated using the Siri (1961) equation. Maximal aerobic capacity was determined in a thermoneutral environment (~22°C, 30% relative humidity (RH)) on a cycle ergometer (Velotron, RacerMate Inc, Seattle, USA). Participants began the test by completing a 5-minute warm-up at 100 W, followed by an incremental increase in workload of 25 W every minute until exhaustion. A silicone facemask connected to an online gas collection system was worn throughout the test to collect expired gases and determine $\dot{V}O_{2peak}$, defined as the highest 30 s value. Peak Power Output (PPO) was determined based on the time completed at the final stage. After sufficient rest a 15 km TT familiarization test was conducted to ensure that the participants could fulfill the requirements of the exercise protocol for the experimental sessions. The TT familiarization was run identical to the experimental sessions, which are explained in greater detail below.

4.2.2 Experimental Sessions

Figure 4 schematically outlines the protocol for the experimental sessions. Participants were instructed to avoid strenuous exercise and caffeine 12 h prior, and

alcohol consumption 24 h prior to each experimental session. Upon arrival, participants voided their bladder and nude body mass was determined. Urine specific gravity (USG) was measured with a refractometer (PAL-10S, Atago, Tokyo, Japan). Participants were considered euhydrated if USG was ≤ 1.020, or else the test was rescheduled. Participants were dressed in cycling jersey, shorts, and shoes, and were instrumented with a 3-lead electrocardiogram (ECG), skin and core thermistors, pulse oximetry, and NIRS probes. They then entered an environmental chamber (Can-Trol Environmental Systems, Markham, Canada) set at 23°C, ~40% RH, airflow ~3.0 $m \cdot s^{-1}$and remained seated in a mesh chair for a 30-minute baseline. After baseline, depending on the experimental session, the environmental chamber either remained at 23°C, ~40% RH, airflow ~3.0 $m \cdot s^{-1}$ for Neutral trials, or was set to 0°C, ~40% RH, airflow ~3.0 $m \cdot s^{-}1$ for the Cold or Cold+Hyper trials. During Neutral trials participants were then set up on the cycle ergometer and fitted with a silicone mask for a 5-minute wash in period. During Cold and Cold+Hyper trials participants put on a hat, mittens, and track pants and sat back down to begin the cooling protocol. Cooling was achieved once participants core temperature decreased by 0.5°C from their baseline core temperature, which took between 40-120 minutes. After cooling participants removed their track pants and were set up on the cycle ergometer and fitted with a silicone mask for a 5-minute wash in period. After wash in, participants completed a 15 km TT. During the TT participants could freely choose their cadence between 60-120 rpm. The only feedback participants received was distance at every 2.5 km mark.

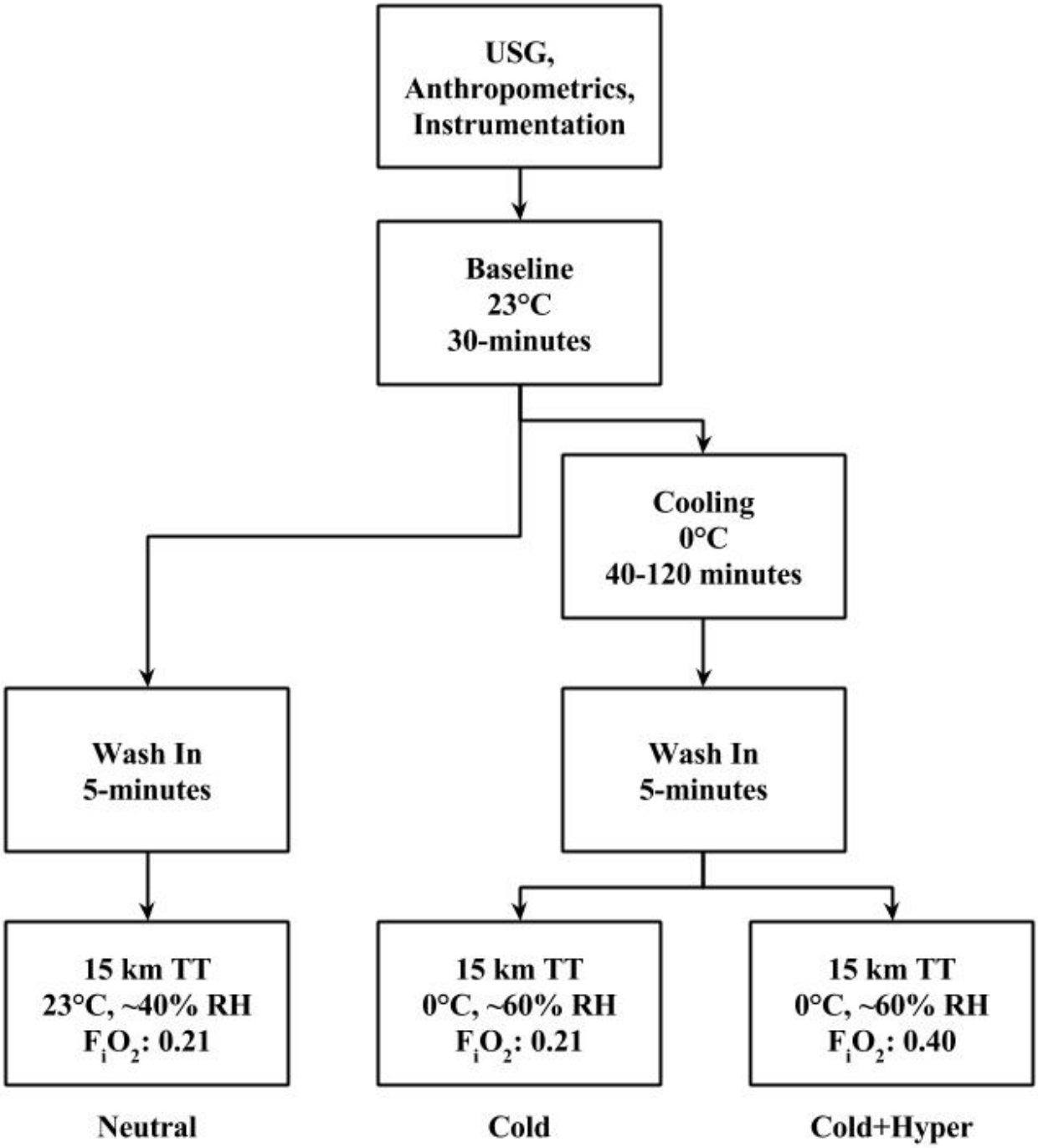


Figure 4 Experimental Protocols. Urine specific gravity (USG); Relative humidity (RH); Time trial (TT); Inspiratory oxygen fraction (F_iO_2).

4.3 Exercise Performance

The cycle ergometer (Velotron, RacerMate Inc, Seattle, USA) was controlled by software (CompuTrainer 3D, RacerMate Inc., Seattle, USA) recording time, power output, cadence, and speed every second. Key variables of interest included: average TT time, power, and cadence across the entire 15 km as well as at each 2.5 km segment.

4.4 Temperature

Rectal temperature was measured with a flexible thermistor (Mon-A-Therm Core, Mallinkrodt Medical, St Louis, USA) inserted 15 cm beyond the anal sphincter, collected through a customized partitional calorimetry unit. Thermocouples (PVC-T-24-190, Omega Environmental Inc., Laval, Canada) were taped (TransporeTM, 3M, St. Paul, USA) to the chest, thigh, upper arm and calf to calculate mean skin temperature ($\overline{T}_{sk}$). $\overline{T}_{sk}$ was calculated using the weighted average of the four thermocouples determined using the Ramanathan (1964) equation:

$$\overline{T}_{sk} = 0.3T_{chest} + 0.3T_{arm} + 0.2T_{thigh} + 0.2T_{leg}$$

All temperature data was continuously sampled at 1 Hz and stored on a personal computer to be analyzed and processed offline using Lab Chart (Version 8, ADInstruments).

4.5 Metabolic Data

Expired gases were collected through a silicone facemask with the exhalation port connected to a metabolic cart (ML206 Gas Analyzer, ADInstruments Inc. Colorado Springs, USA). Expired gas from the breathing apparatus was continuously sampled during the wash in and 15km TT to determine minute ventilation ($\dot{V}_E$; $L \cdot min^{-1}$), and

volume of O_2 ($\dot{V}O_2$ $L \cdot min^{-1}$). Hyperoxic gas (F_iO_2: 0.40) was stored in a Douglas bag and was administered to the breathing apparatus through a valve and tube system. The O_2 concentration administered to the subjects was double blinded for the duration of the study, as the Douglas bag remained outside the chamber and the experimenter inside the chamber was not informed of the gas mixture.

4.6 Heart Rate and S_aO_2

Heart Rate was continuously obtained from R–R intervals using a 3-lead electrocardiogram (Bio Amp, ADInstruments, Colorado Springs CO, USA) and collected using Lab Chart (Version 8, ADInstruments). Arterial oxyhemoglobin saturation (S_aO_2) was measured with pulse oximetry (Bio Amp, ADInstruments, Colorado Springs CO, USA) on the left middle finger of each participant.

4.7 NIRS

Cerebral oxygenation and muscle oxygenation was measured with a three-wave length (775, 810, 850 nm) high temporal resolution near infrared spectroscopy (NIRS) device (NIRO-200, Hamamatsu Photonics, Hamamatsu, Japan). The theory, limitations, and reliability of measurements obtained with NIRS has been previously detailed (Rooks et al., 2010; Scheeren and Schwarte, 2012). The NIRS unit consists of two detector photodiodes and three laser-emitting diodes held 4 cm apart by rubberized shell casings. The probes were attached to the left forehead, approximately 3 cm from the midline and just above the supra-orbital ridge (Miyazawa et al., 2013) and the right vastus-lateralis along the vertical axis of the thigh approximately 10-14 cm from the knee joint (Farra et al., 2017). These measurements were recorded for each participant during the familiarization trial to facilitate accurate replacement for each participant. After

instrumentation, the NIRS probes were set for optimal measurement conditions by commencing the initialization function on the NIRO-200. The zero set procedure was applied to reset oxygenated hemoglobin (O_2Hb), deoxygenated hemoglobin (HHb), and total hemoglobin (tHb) values to an arbitrary zero value. The tissue oxygenation index (TOI) values are not affected by this procedure as TOI is measured in absolute values instead of a change from the arbitrary initial zero value (Ihsan et al., 2013). The intensity of incident and transmitted light was recorded continuously at 10 Hz and, along with the specific extinction coefficients and optical path length, used for online estimation and display of concentration changes (Dlmol/L) in O_2Hb, HHb and tHb according to the Modified-Beer-Lambert law. TOI was measured with the spatial resolved spectroscopy method. Probes were affixed using adhesive tape (Ref 71443-02, Hypafix, Germany) as well as an adhesive plastic layer between the skin to prevent movement and signal contamination from external light sources. O_2Hb is representative of oxygen bound to hemoglobin, while HHb is representative of tissue unbound to hemoglobin. tHb is representative of regional changes in blood volume. TOI is the percentage of O_2Hb/tHb, which indicates the amount of tissue oxygenation.

4.8 Capillary Lactate

In order to analyze blood lactate concentration (YSI 23L Lactate Analyzer, YSI Scientific, Yellow Springs, USA) a capillary blood sample was obtained from the earlobe at rest, pre-exercise, and immediately after the end of exercise.

4.9 Perceptual Scales

Perceptual measures of exercise, thermal comfort and sensation were recorded at baseline, pre-exercise, and every 2.5 km of the TT. RPE was assessed using a 6-20 scale

(Borg, 1982). Thermal comfort (TC) was assessed on a 1 (comfortable) to 4 (very uncomfortable) scale, while thermal sensation (TS) was reported on a 1 (very cold) to 7 (very hot) scale (Gagge et al., 1967).

4.10 Statistical Analysis

Results are presented as the mean ± SD, and the alpha level was set to $p < 0.05$. Normal distribution was assessed by skewness and kurtosis measures and by visual inspection of histograms. All variables were analyzed with a two-way repeated measure ANOVA condition (Neutral, Cold, Cold+Hyper) and time points (Baseline, Cooling, Pre-Exercise, 2.5 km, 5.0 km, 7.5 km, 10 km, 12.5 km, 15 km). A one-way ANOVA was conducted on average TT time, power, and cadence. A paired samples t-test was performed to compare differences between cooling times for Cold and Cold+Hyper Bonferroni post-hoc corrections were performed for multiple comparisons where significant main effects were found. All statistical analyses were performed with GraphPad Prism (version 7, GraphPad Software Inc., La Jolla, CA, USA).

Chapter 5: Results

5.1 Cycling Performance Variables

Mean TT time (Neutral:1479 ± 75s, Cold: 1509 ± 88s, Cold+Hyper: 1482 ± 85s) was faster in Neutral vs. Cold ($p = 0.005$) and Cold vs. Cold+Hyper ($p = 0.006$), while there was no difference in Neutral vs. Cold+Hyper ($p = 0.999$). TT Time during Neutral was faster than Cold ($p \leq 0.005$) at every time point except 12.5 km. TT Time during Cold+Hyper was faster than Cold ($p \leq 001$) from 7.5 km to 15 km and was not different from Neutral ($p \geq 0.356$) at any time point.

Mean power output (Neutral: 260.4± 38.4 W, Cold: 245.8 ± 40.5 W, and Cold+Hyper: 256.2 ± 42.3 W) was higher in Neutral vs. Cold ($p = 0.001$) and Cold+Hyper vs. Cold ($p = 0.044$), while there was no difference in Neutral vs. Cold+Hyper ($p > 0.683$). Power output during Neutral was higher than Cold at all time points except 12.5 km ($p \leq 0.012$). Neutral and Cold+Hyper were not different from each other at any time point during the TT ($p \geq 0.161$). Power output during Cold was lower than Cold+Hyper from 7.5 km to 15 km ($p \leq 0.003$).

Mean cadence (Neutral: 92.6 ± 7.8 RPM, Cold: 89.2 ± 7.3 RPM, Cold+Hyper: 89.8 ± 8.0 RPM) was faster in Neutral than both cold conditions ($p = 0.031$). Cadence was faster than Cold+Hyper ($p \leq 0.021$) but not different from Cold at 2.5 km ($p \geq 0.999$) Cadence during Neutral was faster at all splits from 5 km to the end of the TT compared to Cold and Cold+Hyper ($p \leq 0.002$). Cadence during Cold was not different at any time point compared to Cold+Hyper ($p \geq 0.567$) (Fig. 5).

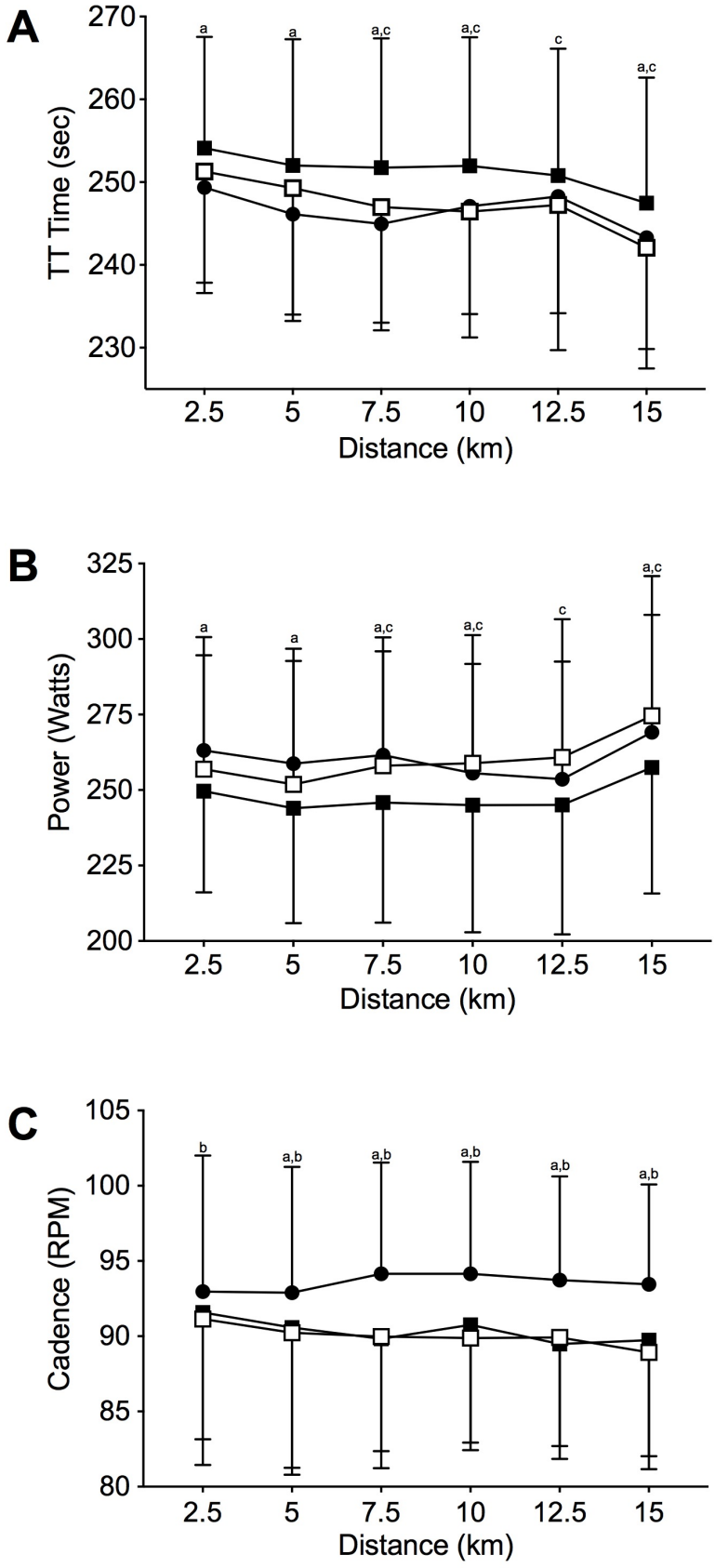


Figure 5 (A) TT Time (B) Power Output. (C) Cadence. Neutral (Closed circles), Cold (Closed squares), Cold+Hyper (Open squares). [a]Neutral significantly different from Cold. [b]Neutral significantly different Cold+Hyper. [c]Cold+Hyper significantly different from Cold.

5.2 Thermal Manipulation

The cooling protocol was successful in eliciting the desired 0.5°C decrease in T_{re} for the two cold conditions (Fig. 6). Cooling times were not statistically different between Cold and Cold+Hyper ($p = 0.734$) taking 86.6 ± 23.9 min and 92.0 ± 19.1 min, respectively. T_{re} was higher ($p \leq 0.016$) in Neutral at all time points compared to Cold and Cold+Hyper. Cold had a higher ($p \leq 0.035$) T_{re} than Cold+Hyper from 2.5 km to 7.5 km. The cooling protocol was also successful in decreasing ($p < 0.001$) $\overline{T}_{sk}$ for the two cold conditions compared to Neutral (Fig. 6). In Cold and Cold+Hyper $\overline{T}_{sk}$ started at 31.1 ± 0.6°C and 30.8 ± 0.8°C and decreased to 21.8 ± 2.2°C and 20.8 ± 2.1°C, respectively. Cold had a higher ($p \leq 0.020$) $\overline{T}_{sk}$ than Cold+Hyper from 2.5 km to 10 km.

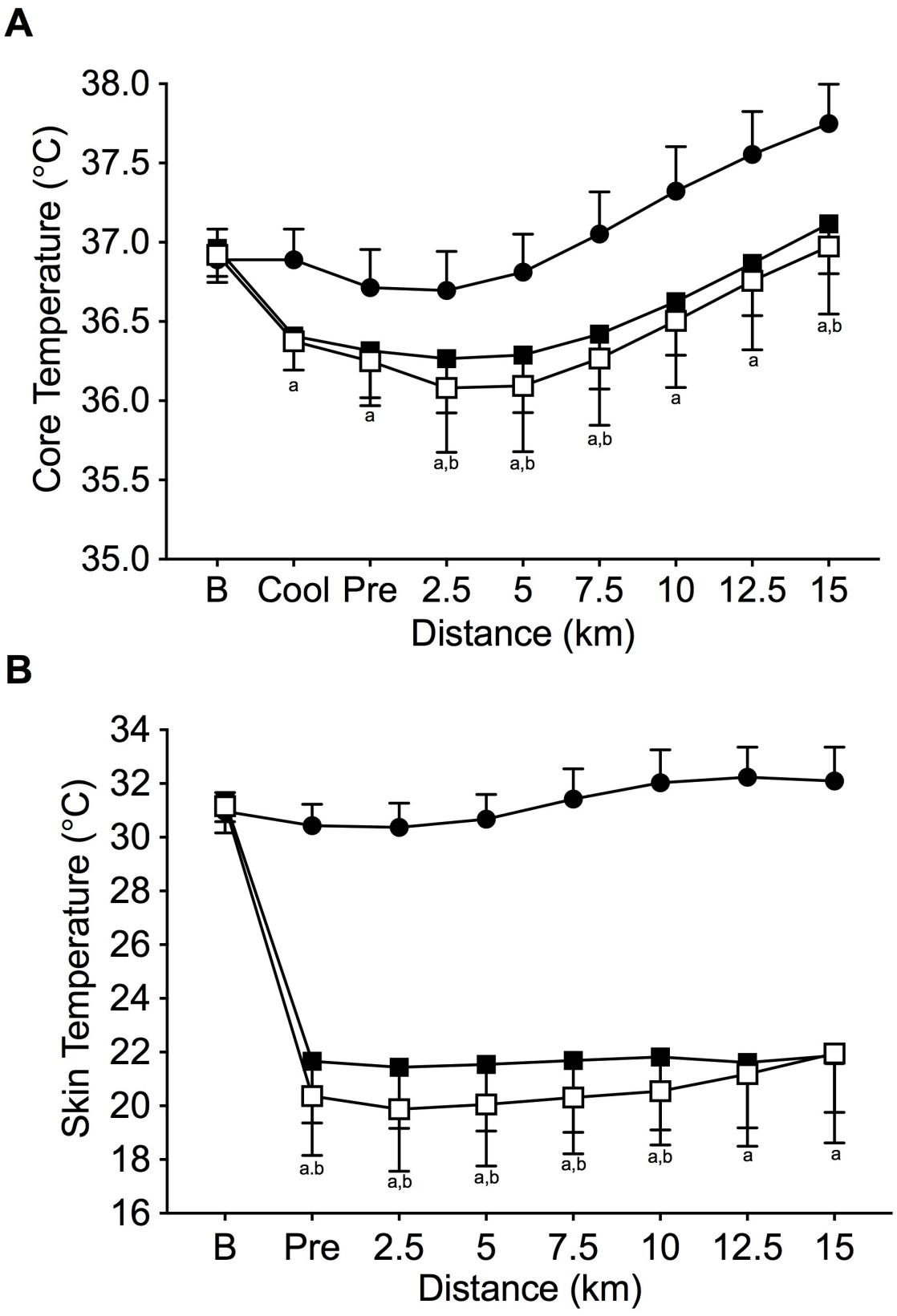


Figure 6 (A) Core temperature. (B) Skin temperature. Neutral (Closed circles), Cold (Closed squares), Cold+Hyper (Open squares). [a]Cold and Cold+Hyper significantly different from Neutral. [b]Cold significantly different from Cold+Hyper.

5.3 Hyperoxia Manipulation

There was no difference ($p > 0.999$) in S_aO_2 at baseline in any of the conditions. Pre-exercise S_aO_2 was not different in Neutral vs. Cold ($p = 0.704$) or Neutral vs. Cold+Hyper ($p > 0.549$), but was different for Cold vs. Cold+Hyper ($p = 0.037$). Hyperoxia improved Cold+Hyper S_aO_2 ($p < 0.001$) compared to Neutral and Cold conditions from 2.5 km to the end of the TT (Fig. 7).

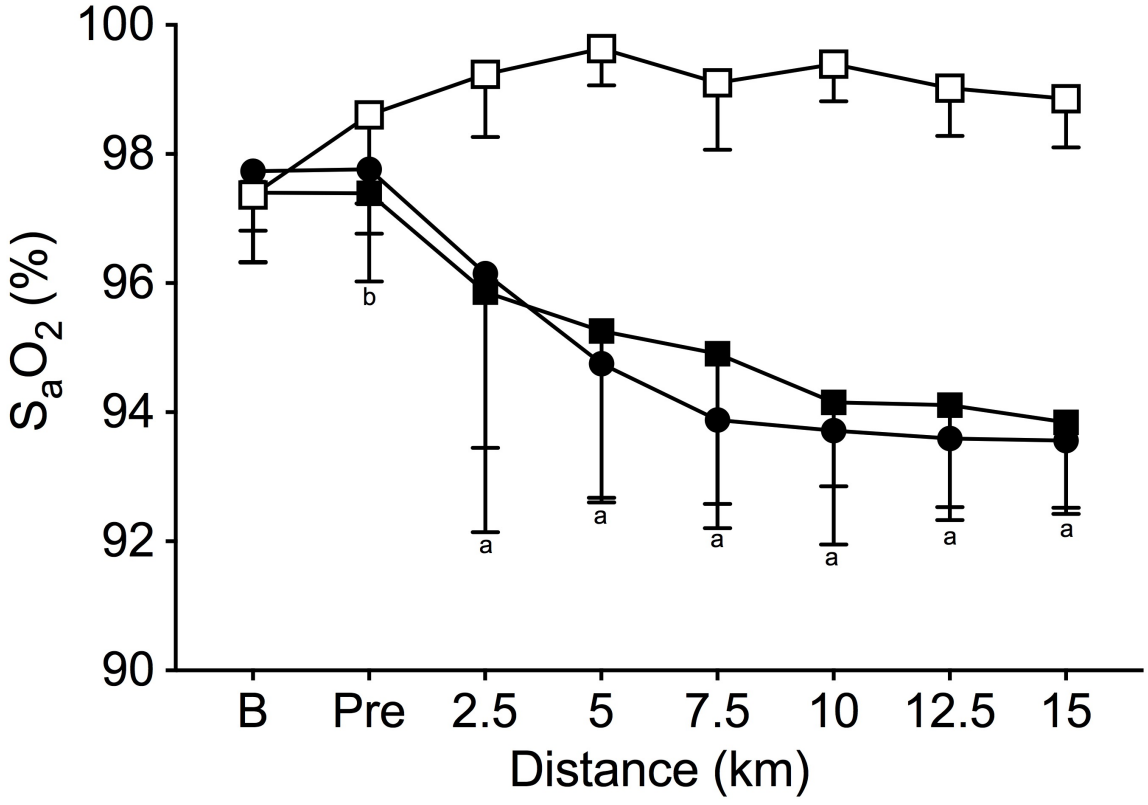
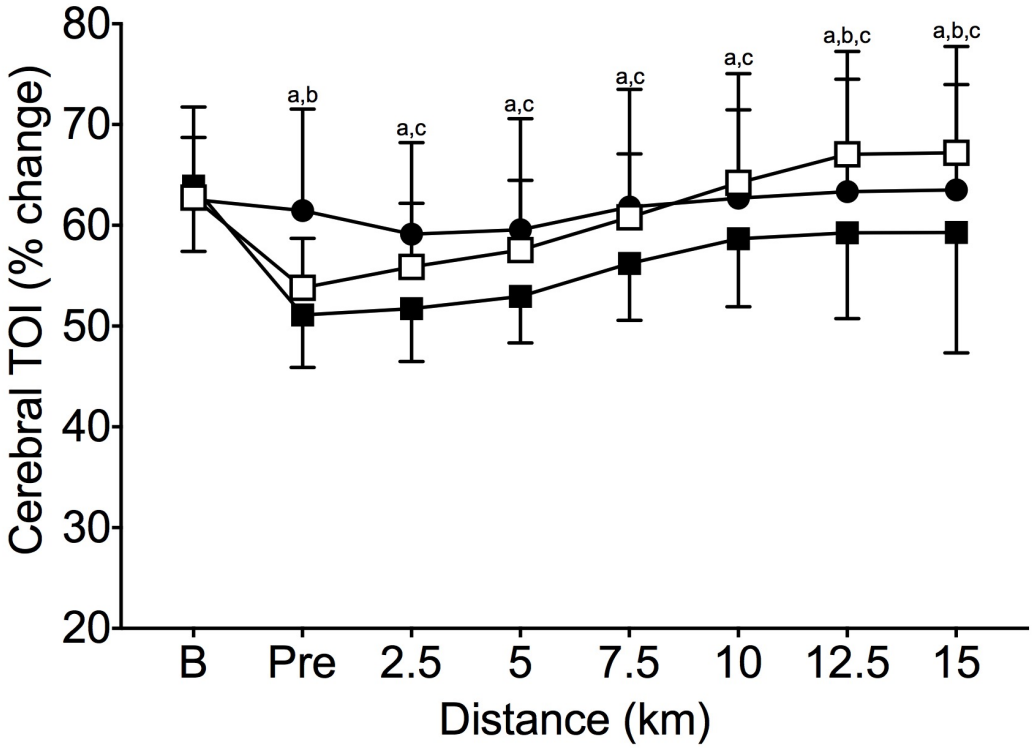


Figure 7 Arterial oxygen saturation. Neutral (Closed circles), Cold (Closed squares), Cold+Hyper (Open squares). [a]Neutral and Cold significantly different from Cold+Hyper. [b]Cold significantly different from Cold+Hyper.

5.4 NIRS

Cerebral TOI was not different at baseline in any of the conditions ($p \geq 0.999$). Cerebral TOI in Neutral was higher than Cold at every time point after baseline ($p \leq 0.011$). Neutral was higher than Cold+Hyper at pre-exercise ($p \leq 0.001$). Neutral and Cold+Hyper were not different from 2.5 km to 10 km ($p \geq 0.492$), while Neutral was lower than Cold+Hyper from 12.5 km to 15 km ($p \leq 0.033$). Cold and Cold+Hyper were not different from each other at pre-exercise ($p \geq 0.188$). Cold was lower than Cold+Hyper from 2.5 km to the end of the TT ($p \leq 0.013$) (Fig. 8).

Muscle TOI was not different at baseline in any of the conditions ($p > 0.999$). Muscle TOI in Neutral was higher than Cold and Cold+Hyper at pre-exercise ($p < 0.001$). Cold was not different to Cold+Hyper pre-exercise ($p = 0.053$). Neutral was higher than Cold from 5 km to 7.5 km ($p \leq 0.036$), while there was no difference between Neutral and Cold at 2.5 km and from 10 km to the end of the TT ($p \geq 0.104$). Neutral was not different from Cold+Hyper from 2.5 km to the end of the TT ($p \geq 0.476$). Cold was lower than Cold+Hyper from 2.5 km to the end of the TT ($p \leq 0.046$) (Fig. 8).



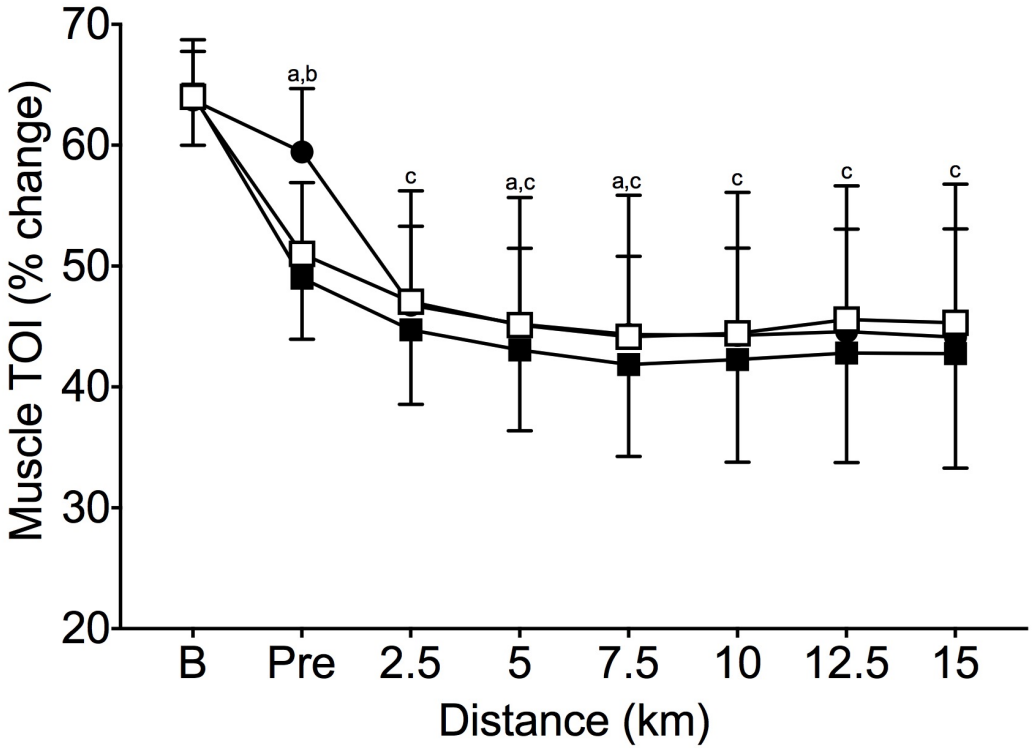


Figure 8 (A) Cerebral TOI. (B) Muscle TOI. Neutral (Closed circles), Cold (Closed squares), Cold+Hyper (Open squares). [a]Neutral significantly different from Cold. [b]Neutral significantly different Cold+Hyper. [c]Cold+Hyper significantly different from Cold.

5.5 Metabolic Variables

VO_2 pre-exercise was lower in Neutral compared to both cold conditions ($p < 0.001$). Cold was also lower than Cold+Hyper pre-exercise ($p < 0.001$). VO_2 at 2.5 km in Neutral was higher than Cold ($p < 0.001$), but not different than Cold+Hyper ($p \geq 0.999$). Cold was also lower than Cold+Hyper at 2.5 km ($p < 0.001$). Neutral was higher than both cold conditions from 5 km to 15 km ($p < 0.001$). Cold in not different from Cold+Hyper at 5 km ($p = 0.222$). Cold is lower than Cold+Hyper from 7.5 km to 15 km ($p < 0.001$) (Figure 9).

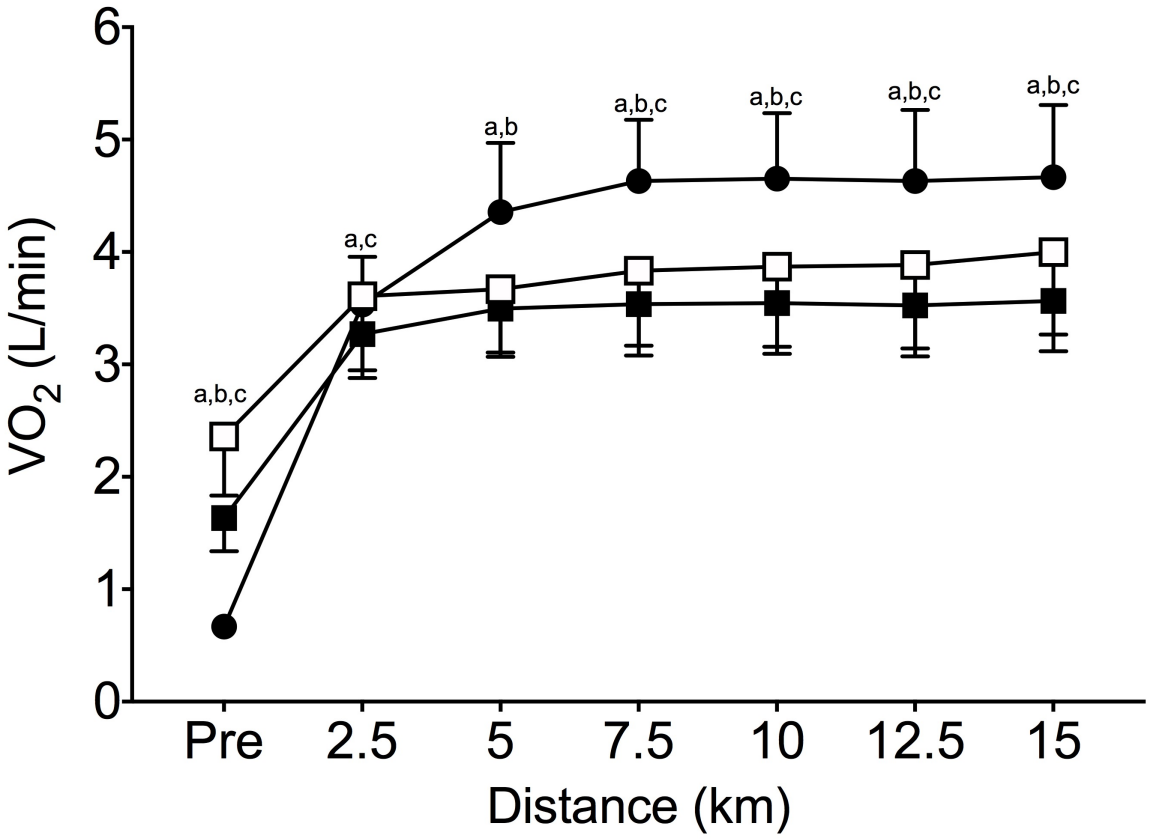


Figure 9 VO_2. Neutral (Closed circles), Cold (Closed squares), Cold+Hyper (Open squares). [a]Neutral significantly different from Cold. [b]Neutral significantly different Cold+Hyper. [c]Cold+Hyper significantly different from Cold.

Minute ventilation for Neutral pre-exercise was lower ($p < 0.001$) than both cold conditions. Neutral was not different from Cold ($p = 0.394$), but was higher than Cold+Hyper ($p = 0.008$) at 2.5 km. Cold was not different from Cold+Hyper at 2.5 km ($p = 0.501$). Neutral was higher compared to Cold and Cold+Hyper from 5 km to 15km ($p < 0.001$) (Fig. 10).

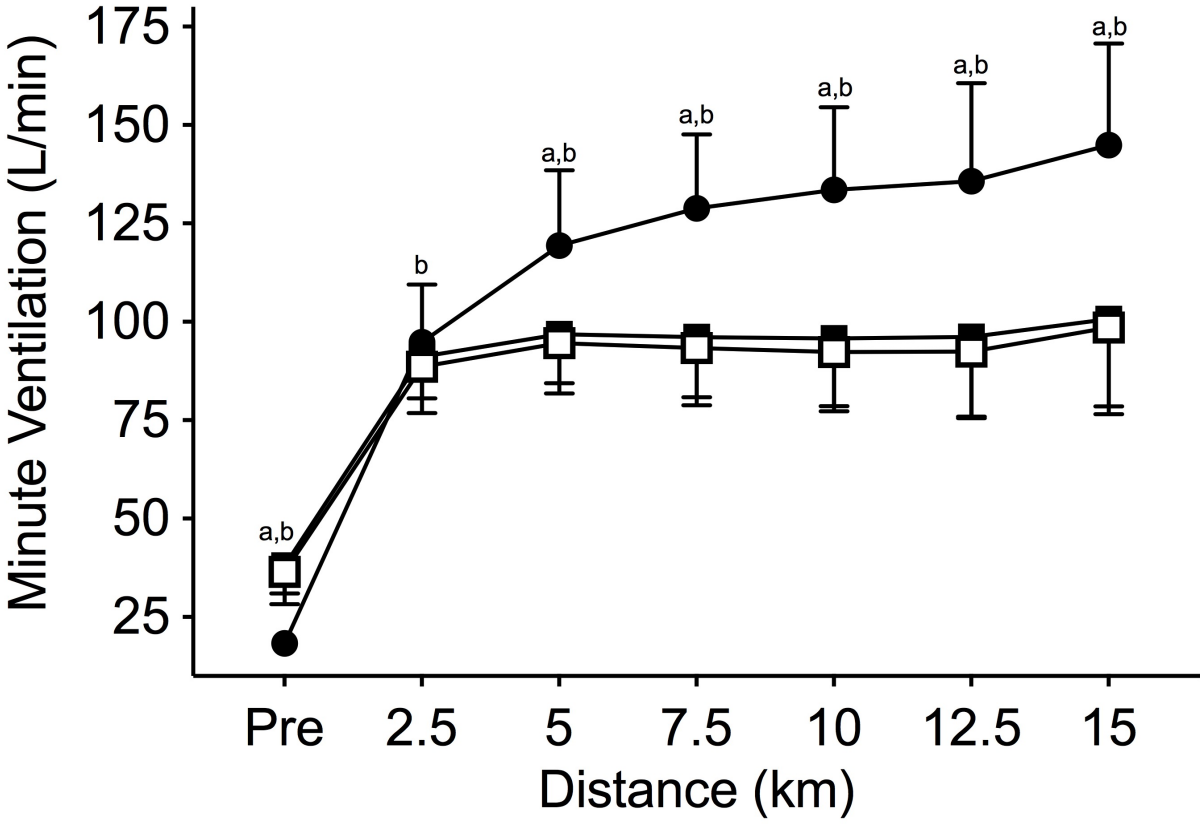


Figure 10 Minute ventilation. Neutral (Closed circles), Cold (Closed squares), Cold+Hyper (Open squares). [a]Neutral significantly different from Cold. [b]Neutral significantly different Cold+Hyper.

5.6 Lactate

Lactate at baseline and pre-exercise were not different between any condition ($p \geq 0.306$). Neutral had a higher lactate than Cold and Cold+Hyper ($p < 0.001$). Cold had a higher lactate than Cold+Hyper ($p = 0.010$) (Fig. 11).

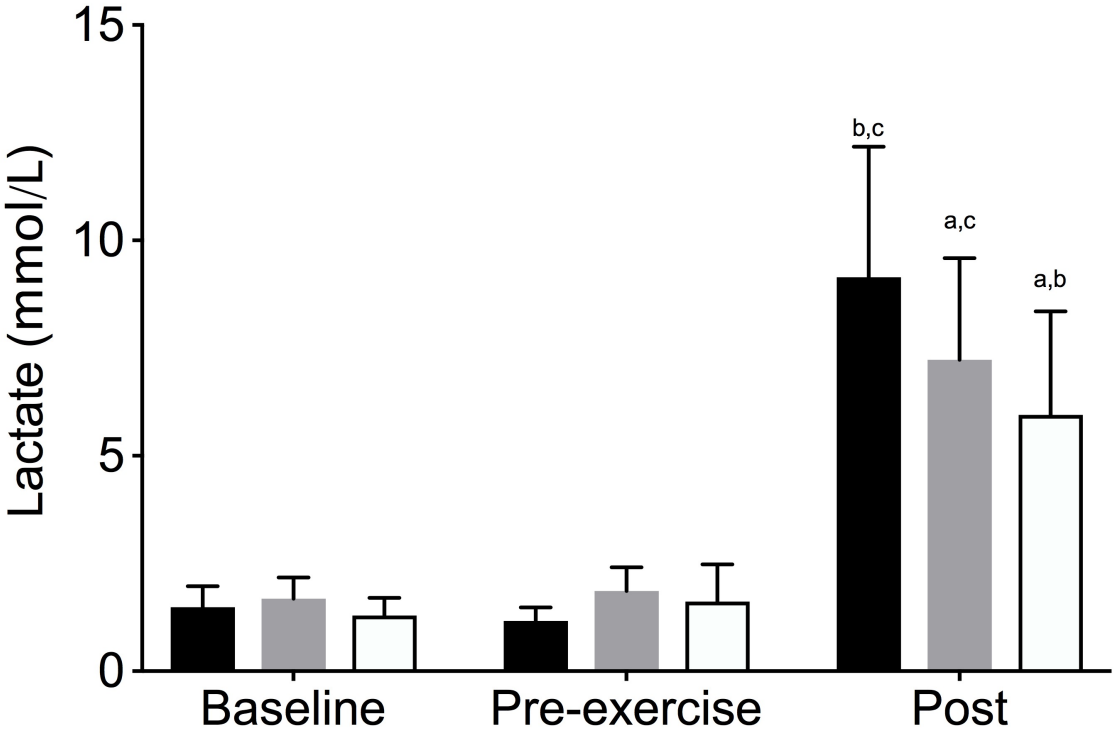


Figure 11 Capillary lactate. Neutral (Black), Cold (Gray), Cold+Hyper (White). [a]Significantly different from Neutral. [b]Significantly different from Cold. [c]Significantly different from Cold+Hyper.

5.7 Cardiovascular

Heart rate was not different between any of the conditions at baseline ($p > 0.999$). Pre-exercise heart rate was higher in Cold and Cold+Hyper compared to Neutral ($p < 0.001$). Cold and Cold+Hyper were not different pre-exercise ($p = 0.544$). Heart rate was higher in Neutral across all time points after pre-exercise compared to Cold and Cold+Hyper ($p \leq 0.002$). Cold was not different from Cold+Hyper at any point during the TT ($p > 0.999$) (Fig. 12).

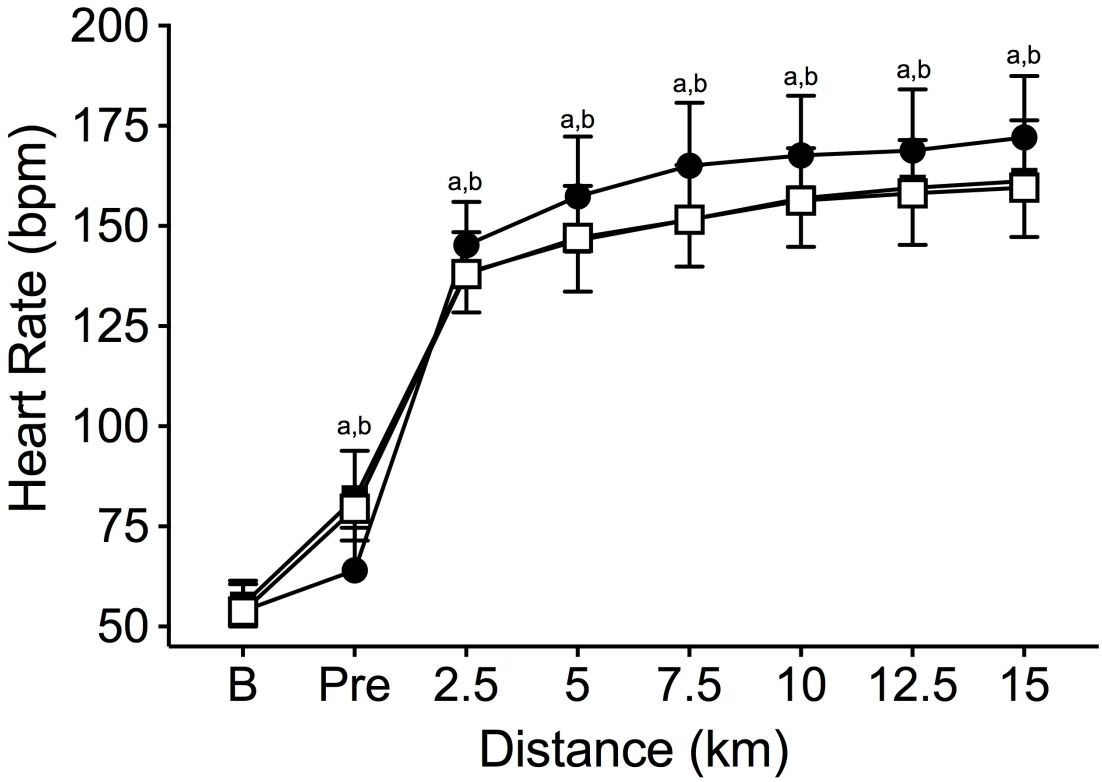


Figure 12 Heart rate. Neutral (Closed circles), Cold (Closed squares), Cold+Hyper (Open squares). [a]Neutral significantly different from Cold. [b]Neutral significantly different Cold+Hyper. [c]Cold+Hyper significantly different from Cold.

5.8 Perceptual Scales

RPE was not different in any of the conditions ($p = 0.182$). TS was not different in any condition at baseline ($p \geq 0.308$). TS during Neutral was warmer at every time point from pre-exercise onwards compared to Cold and Cold+Hyper ($p < 0.001$). TS between Cold and Cold+Hyper was not different at any time point ($p > 0.999$). TC was not different in any condition at baseline ($p \geq 0.852$). TC during Neutral was better at each time point after baseline until 10 km ($p \leq 0.004$). TC during Neutral was not different than Cold at 10 km ($p = 0.157$, but was different compared to Cold+Hyper ($p = 0.011$). TC was not different between any condition from 12.5 km to 15 km ($p \geq 0.157$) (Fig 13).

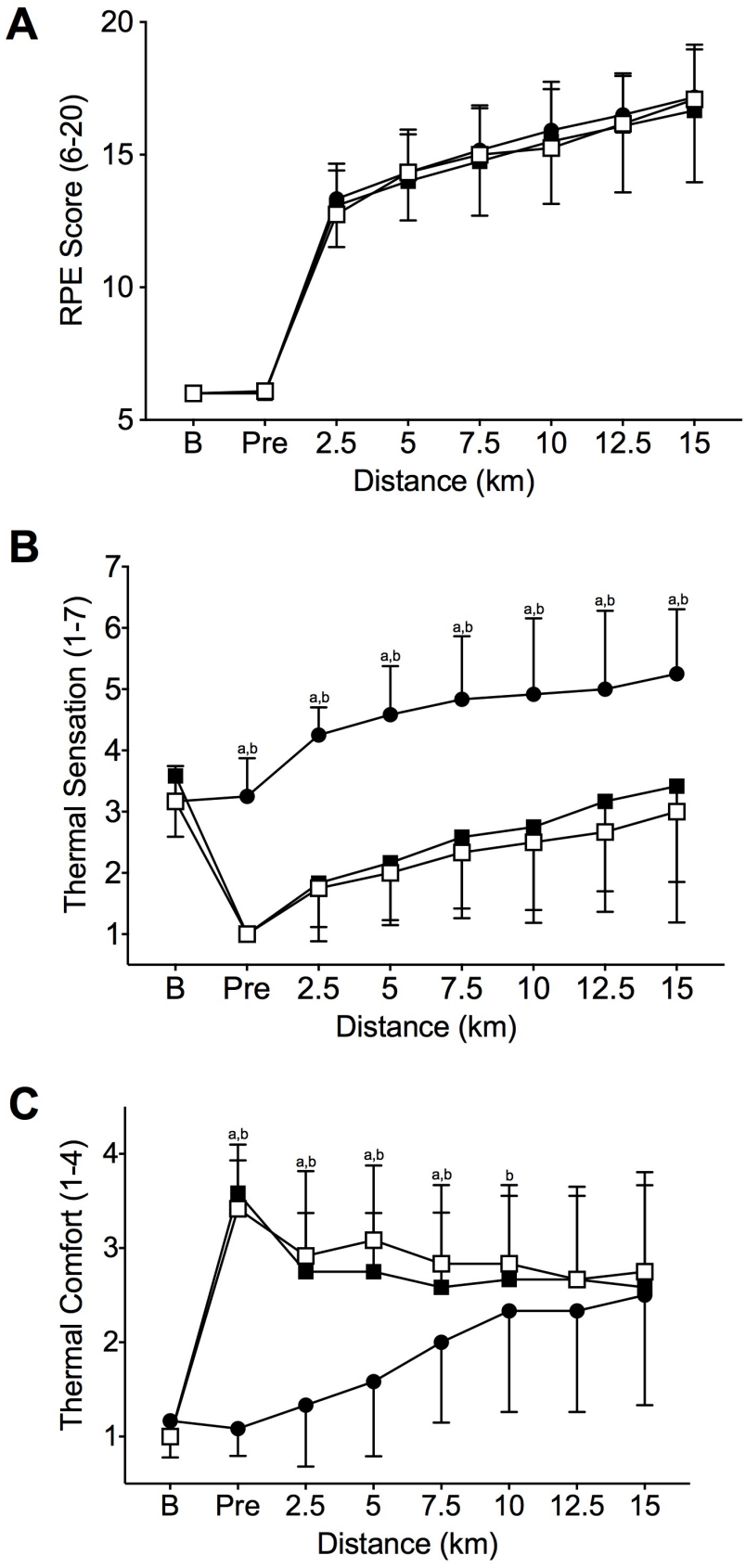


Figure 13 A) RPE, B) TS, C) TC. Neutral (Closed circles), Cold (Closed squares), Cold+Hyper (Open squares). [a]Neutral significantly different from Cold. [b]Neutral significantly different Cold+Hyper. [c]Cold+Hyper significantly different from Cold.

Chapter 6: Discussion

The primary objectives of this study were to first determine if hyperoxia can improve exercise performance in mildly-cooled individuals and then secondly, to investigate potential mechanisms for improvement such as changes in cerebral and muscle oxygenation. Passive cooling in 0°C occurred to reduce T_{re} by 0.5°C from baseline levels in the two cold conditions as a means to reduce exercise performance during a 15 km TT. The administration of hyperoxia (F_iO_2 0.40) in Cold+Hyper was then used to see if performance could be improved compared to Cold. Similar reductions in T_{re} and $\overline{T}_{sk}$ throughout Cold and Cold+Hyper suggests that hyperoxia had no effect on thermogenic responses. Overall, performance was improved with hyperoxia as TT time for Cold+Hyper was faster than Cold, while no difference was found compared to Neutral (Neutral: 1479 ± 75s, Cold: 1509 ± 88s, Cold+Hyper: 1482 ± 85s). The improvement in TT time was reflected by changes in power output where Cold was significantly lower than Neutral throughout the TT; in contrast, Cold+Hyper was not different from Neutral for most of the TT. Hyperoxia restored cerebral oxygenation in Cold+Hyper to Neutral levels by 2.5 km, following the same pattern as power output. Muscle oxygenation in both Cold and Cold+Hyper were similar to Neutral throughout the TT, but muscle oxygenation in Cold+Hyper was improved compared to Cold. Since cadence was similar between Cold and Cold+Hyper, the difference in power output came through greater torque, implying greater neuromuscular recruitment possibly from the improved O_2 availability allowing for improved aerobic metabolism throughout the body.

The reduction in exercise performance in cold may come partially through the increased metabolic and muscular demands from moderate shivering. Using a similar

protocol of passive cooling to -0.5°C from baseline, Gagnon et al. (2014) demonstrated that treadmill speed at a constant 50 and 70% VO_{2peak} was lower, implying that extra metabolic demand was required to sustain shivering. In our TT protocol, where self-pacing was possible but at a much higher intensity of approximately 80-85% VO_{2peak}, both power output and VO_2 were decreased for Cold throughout the entire TT compared to Neutral. With the addition of hyperoxia in Cold+Hyper, T_{re} and $\overline{T}_{sk}$ along with VO_2 remained reduced at similar levels as Cold throughout the TT, yet power output was higher than Cold and restored to Neutral levels from 2.5 km onwards. In addition, perceptual scales of TS and TC did not differ between the two cold conditions at any time point either, indicating the use of hyperoxia did not alter thermal tolerance between the cold conditions. This combination implies that hyperoxia did not improve exercise capacity in the cold through a thermogenic benefit or reduction in shivering demands.

The primary proposed mechanism for hyperoxia's benefit is through improved O_2 availability (Welch, 1981), but whether this occurs despite the systemic vasoconstriction from mild cooling is unknown. Hyperoxia was able to maintain S_aO_2 in Cold+Hyper at ~99% throughout the entire duration of the TT, while Neutral and Cold were reduced by ~4-5% and remained there throughout the entire TT (Fig. 7). The maintenance in S_aO_2 at baseline levels during Cold+Hyper implies that any reductions in blood flow as a result of vasoconstriction from the cold were compensated for by maintained O_2 availability in the blood for extraction. Stellingwerff et al. (2006) showed that hyperoxia in a thermoneutral environment reduced lactate concentrations as a result of improved aerobic metabolism, which could explain our results. Reduced rates of various metabolites are of great importance since group III and IV muscle afferents innervate free nerve endings

distributed widely throughout the muscle (Amann et al. 2006a). Metabolic byproducts of muscular contractions such as H^+ and P_i have been shown to increase the spontaneous discharge of both group III and IV afferents (Amann et al., 2011), therefore sending inhibitory feedback to the central nervous system and reducing central motor drive (Amann et al., 2006; Nybo and Rasmussen, 2007; Amann and Calbet, 2008). Thus, reduced lactate concentrations found in the present study during Cold+Hyper compared to Neutral and Cold could have contributed to the decreased biochemical and physiological disturbances to homeostasis by allowing greater reliance on aerobic metabolism, thus allowing for improved exercise performance.

The secondary objective of this study was to determine potential mechanisms that could explain an improvement in performance, therefore we examined changes in both cerebral and muscle oxygenation. Both cerebral and muscle oxygenation were improved in Cold+Hyper compared to Cold, however the differences in cerebral oxygenation were larger than those in muscle oxygenation when hyperoxia was administered. Therefore, we suggest that potential mechanisms explaining enhance performance are more likely related to changes in cerebral rather than muscle oxygenation. The restoration of power output in Cold+Hyper to Neutral levels from 2.5-15 km of the TT coincided with a restoration of cerebral oxygenation, such that O_2 availability in the CNS may play a critical role in influencing central motor drive. Amann et al. (2007) showed that physical fatigue during acute exposure to severe hypoxia (F_iO_2: 0.10) was largely influenced by central factors, since a rapid switch to hyperoxia (F_iO_2: 0.60) improved cerebral oxygenation and prolonged TTE in the absence of a critical level of peripheral muscle fatigue. Similarly, Subudhi et al. (2008) showed that hyperoxia (F_iO_2: 0.60) administered

at maximal exertion during acute hypoxia increased cerebral oxygenation above resting values and increased pedal cadence during a graded exercise test, allowing a similar maximal work rate to be achieved compared to normoxia. In both studies, the administration of hyperoxia alleviated cerebral hypoxemia, extending exercise tolerance and reaching similar maximal work rates as achieved in normoxia. While the present study was not done in a hypoxic environment, greater cerebral deoxygenation also occurred at the beginning of exercise in Cold and Cold+Hyper compared to Neutral. Thus, we argue that any cerebral hypoxemia occurring initially in Cold+Hyper was completely alleviated as shown by the restoration of cerebral oxygenation compared to Cold by 2.5 km. Together, these studies suggest that there may be a hypoxemia-sensitive up or down regulation of central motor drive outside of any peripheral muscle fatigue and its associated afferent feedback.

Limitations

Healthy trained cyclists were used to determine if hyperoxia can improve exercise performance in mildly cooled individuals. Therefore, results from this study may not be generalizable to less fit populations, occupational workers, or even different types of athletes. While the study only targeted trained cyclists, a diverse age group (22-48) was used, which allows us to determine that the administration of hyperoxia could work on a large spectrum of age. Future studies should examine the role of hyperoxia on multiple athletic and occupational populations.

We acknowledge that placing the cerebral NIRS on the left frontal lobe to determine cerebral oxygenation may not be reflective of global cerebral oxygenation, as more active regions of the brain may receive a greater proportion of blood flow (Delp et

al., 2001). We also acknowledge that the left frontal lobe and right vastus lateralis are two regional measurements of the body, so results may not represent changes occurring in the entire body. Future studies should examine multiple regions of cerebral and muscle oxygenation in order to gain a clear understanding of the cerebral and muscular response to hyperoxia administered in mildly cooled individuals.

We also acknowledge that there are other measures that can be used to assess both central and peripheral fatigue such as the use of EMG with transcranial magnetic stimulation and peripheral nerve stimulation. However, it was not the purpose of this study to identify whether central or peripheral mechanism were responsible for improvements in performance. Rather we sought to shed light on whether performance can be improved with the administration of hyperoxia in mildly cooled individuals while investigating potential mechanisms if an improvement was found.

Future Directions

The present study has presented the following questions that warrant further research:

1) Does the administration of hyperoxia in mildly cooled individuals primarily influence central motor drive as a result of central or peripheral fatigue? To assess this surface electromyography could be used during a TT while magnetic stimulation could be used prior to and after exercise.

2) Does hyperoxia administered in normoxia and hypoxia at exhaustion allow exercise performance to continue in mildly cooled individuals as a result of improved cerebral or muscle oxygenation? To assess this, individuals would be administered

hyperoxic gas at exhaustion to see if there is a link between cerebral and muscle oxygenation.

Conclusion

In summary, this study set out to determine if hyperoxia can improve dynamic exercise performance in mildly-cooled individuals, as well as investigate potential mechanisms for improvement. We conclude that hyperoxia (F_iO_2: 0.40), when administered in mildly cooled individuals can be used as an ergogenic aid as shown by improved TT times and increased power output. Our results suggest that this improvement is mainly due to the increased O_2 availability as shown by a maintained S_aO_2 during Cold+Hyper compared to Neutral and Cold. While the main purpose of the study was to look at changes in performance, our results suggest that cerebral oxygenation could be a potential mechanism behind improved performance. Since hyperoxia was able to restore cerebral oxygenation in Cold+Hyper to Neutral levels for most of the 15 km cycling time trial, the reversal of cerebral deoxygenation could have prevented any potential cerebral hypoxemia that may have occurred due to increased desaturation seen in both cold conditions. Further research should investigate the contributions of cerebral and muscle oxygenation as well as central and peripheral fatigue and their role on performance in mildly cooled individuals.

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Chapter 8: Appendix A

Brock University
Research Ethics Office
Tel: 905-688-5550 ext. 3035
Email: reb@brocku.ca

Bioscience Research Ethics Board

Certificate of Ethics Clearance for Human Participant Research

DATE:	9/21/2016		
PRINCIPAL INVESTIGATOR:	CHEUNG, Stephen - Kinesiology		
CO-INVESTIGATOR(S):	Brian Roy (broy@brocku.ca); Neil Eves (neil.eves@ubc.ca)		
FILE:	16-017 - CHEUNG		
TYPE:	Masters Thesis/Project	STUDENT:	Steven Ferguson
		SUPERVISOR:	Stephen Cheung

TITLE: The effects of hyperoxia on exercise performance in the cold

ETHICS CLEARANCE GRANTED

Type of Clearance: NEW Expiry Date: 9/29/2017

The Brock University Bioscience Research Ethics Board has reviewed the above named research proposal and considers the procedures, as described by the applicant, to conform to the University's ethical standards and the Tri-Council Policy Statement. Clearance granted from **9/21/2016** to **9/29/2017**.

The Tri-Council Policy Statement requires that ongoing research be monitored by, at a minimum, an annual report. Should your project extend beyond the expiry date, you are required to submit a Renewal form before 9/29/2017. Continued clearance is contingent on timely submission of reports.

To comply with the Tri-Council Policy Statement, you must also submit a final report upon completion of your project. All report forms can be found on the Research Ethics web page at http://www.brocku.ca/research/policies-and-forms/research-forms.

In addition, throughout your research, you must report promptly to the REB:

a) Changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
b) All adverse and/or unanticipated experiences or events that may have real or potential unfavourable implications for participants;
c) New information that may adversely affect the safety of the participants or the conduct of the study;
d) Any changes in your source of funding or new funding to a previously unfunded project.

We wish you success with your research.

Approved: [redacted]
Sandra Peters, Chair
Bioscience Research Ethics Board

Note: Brock University is accountable for the research carried out in its own jurisdiction or under its auspices and may refuse certain research even though the REB has found it ethically acceptable.

If research participants are in the care of a health facility, at a school, or other institution or community organization, it is the responsibility of the Principal Investigator to ensure that the ethical guidelines and clearance of those facilities or institutions are obtained and filed with the REB prior to the initiation of research at that site.